Diagnosis of the Sarcoma by a Skin-Test.

By

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In taking advantage of an opportunity reported elsewhere,[1] a watery extract was prepared from sarcomatous mass, almost pure, and injected intracutaneously to the patients suffering from the sarcoma, carcinoma or various diseases other than any neoplasma. As the results, in the case of sarcoma the skin reaction took place almost invariably while no reaction occurred in the patients suffering from the carcinoma.

Experimental.

Material: Spindle cell sarcoma originated from the great omentum. It was of consistence of the brain mass, and occupied the abdominal cavity in full, so that we thought the latter was filled with the ascites, but get the white, soft mass instead of the watery by puncture while the patient was alive. A portion, about 150 grm., of the mass floating in the abdominal cavity at autopsy, was used for the present investigation.

Extraction: The mass was placed in 95% alcohol for a few days, being shaken from time to time. Alcohol then was discarded, the remaining mass was dried and powdered in a mortar. 2% watery emulsion was made therefrom, shaken thoroughly. To the supernatant liquid, separated by centrifugalization, about 5 volume alcohol was added, and shaken thoroughly. The precipitation, separated by centrifugalization, was washed and dehydrated with alcohol and ether, and then dried in the vacuum exsiccator, followed by powdering. The powder was dissolved in the saline solution, the carbolic acid being added to 0.5% for preservation.

Dosis, estimation: 0.1 cc. emulsion was intracutaneously injected on the palmar face of the upper arm, and the same quantity of 0.5% carbolic acid solution on the other side as the control. At first 0.05 cc. and 0.2 cc. emulsion were tried too, with similar outcome.

In loco the redness, induration and bubbles develop as a consequence. At 1, 2, 3, 4, 5, 24, 48 & 72 hours after the injection they were noted.

The largeness of induration was chiefly marked, the redness and bubbles formation being taken into account also.

On the base, given below, the induration, the diameter of which exceeds 1 cm. at the end of 48 hours after the injection, is taken unconditionally as a positive reaction for the sarcoma. And if the induration, with the diameter a little less than 1 cm. be combined with bubbles, clearly visible, it is taken as a positive case.

(1) In this skin-test, the induration has a boundary clearly determinable than that of the redness, and swells nodularly, in comparison with that was brought about by the control test with carbolic acid or the Mantoux-Mendel's.

(2) It is commonly difficult to say of the width of the redness because of the irregularity of expansion and of indistinctness of its boundary.

(3) The largeness of the induration, determined 24 hours after the injection and 48 hours, i.e. another 24 hours later, practically the same each other in the sarcoma patients only.

In all other cases, that is, even in the patients suffering from the carcinoma and other neoplasma, the induration is to be found as being reduced undoubtedly, when measured 48 hours after the injection.

(4) The observation for a short time such as 1–5 hours cannot be taken as sufficiently adequate. The carbolic acid only, for example, is able to produce the reaction of similar degree if the observation is limited within such a length of time as 5 hours. At 24 hours after the injection no trace of induration exists. In the cases of sarcoma the induration was invariably found 72 hours after the injection as still existing, while no or almost no induration was found at all 24 hours after the extract injection in the carcinoma cases.

In the skin reaction due to the carcinoma extract, which gives a promissing data in the hands of Seo[2] and Matsubara,[3] the latter author relied on observation extending for some hours to one hour only. The extract of mouse sarcoma, it may be here added in passing, can evoke also the skin reaction in patients suffering from carcinoma and in pregnant women.[3d]

Results.

It must be noticed first that the extract was applied here to the patients, usually before identificating the nature of tumor.

Healthy persons: No positive case in 10 persons. (24 hour test gave 5 positive reaction (induration with over 1 cm. diameter))

Sarcoma patients: In toto 12 cases were examined; 11 cases positive, with 1 negative case. All the cases, which gave positive skin reaction in our hands, 11 in number, were proved histologically as the sarcoma. With a single case, which was diagnosed as the sarcoma clinically, especially by the beneficial effect of X-ray, the induration did not exceed 1 cm. at last. The observation was not extended to 72 hours; in fact this case was investigated at the very beginning of the present observa-

TABLE I.

Sarcoma Patients.

No.	Sex & Age	Clinical diagnosis	24 hrs.	Judgment	48 hrs.	Judgment
1	♂ 21	Mediastinal sarcoma	R(++) I(+) 9× 9 B(+) 4× 4	+	R(+) I(+) 7× 7 B(+) 3× 3	+
2	♂ 56	R. cervical lympho-sarcoma	R(+) I(+) 8×10 R(++) I(+) 3× 3	−	R(±) I(+) 6× 6 R(+) I(+) 3× 3	−
3	♀ 27	Lympho-sarcomatosis	R(+) I(+) 32×32 R(++) I(+) 7× 7 B(+) 3× 3	+	R(+) I(+) 18×22 R(++) I(+) 6× 7 B(+) 2× 3	+
4	♀ 13	Ovarial sarcoma	R(+) I(±) 22×33 R(++) I(+) 5× 6 B(+) 2× 2	+	R(+) I(+) 7× 8 B(+) 2× 3	+
5	♂ 52	Osteosarcoma of r. humerus	R(+) I(+) 25×25 R(++) I(+) 7×10 B(+) 2× 3	+	R(+) I(+) 14×20 R(++) I(+) 9×10 B(+) 3× 4	+
6	♂ 18	Osteosarcoma of l. humerus	R(+) I(+) 14×15 R(+) I(++) 7× 7 B(+) 3× 3	+	R(+) I(+) 10×11 B(+) 2× 3	+
7	♂ 20	Mandibullar sarcoma	R(±) I(+) 22×25 R(+) I(+) 7× 7 B(+) 2× 3	+	R(+) I(+) 12×14 R(+) I(++) 6× 7 B(+) 3× 4	+
8	♂ 50	Cervical lymphosarcoma	R(+) I(−) 25×25 R(++) I(±) 10×12 R(++) I(+) 5× 6	+	R(+) I(±) 11×13 R(++) I(+) 5× 5 B(+) 2× 3	+
9	♂ 31	Lymphosarcoma in fovea jugul. & axillary fossa	R(−) I(+) 20×20 R(+) I(+) 8× 8 B(+) 3× 3	+	R(−) I(+) 15×15 R(++) I(++) 8×10 B(+) 4× 4	+
10	♂ 66	Fibrosarcoma in larynx	R(+) I(+) 9×10 B(+) 5× 5	+	R(+) I(+) 6× 6 B(+) 3× 3	+
11	♀ 67	Lymphosarcoma in r. tonsil	R(+) I(+) 18×18 R(++) I(+) 6× 6	+	R(++) I(+) 7× 7 B(+) 2× 3	+
12	♂ 50	Pelvis sarcoma	R(±) I(+) 10×10 B(+) 2× 2	+	R(±) I(+) 10×10 B(+) 2× 2	+

R: Redness I: Induration B: Bubbles Numerals: mm.

tion when it was not sufficiently long. The data are epitomized in the accompanying table.

Carcinoma cases: Among 33 cases (thyroid 1, lung 3, esophagus 4, stomach 18, liver 2, pancreas 2, rectum 2, uterus-metastasis only 1) only 2 cases gave us positive reaction against the sarcoma extract. The following two cases are of interesting to note: Each case of the lung and

the pancreas was diagnosed clinically as the sarcoma, and in fact the latter as so even at autopsy; but the skin reaction was always negative, against our expectation. And in reality microscopical examination finally uncovered its character; they were the cancer. Our skin test thus stands on a firm ground.

All the patients with sarcoma and the majority of the carcinoma patients originated from the cliniques of Surgery, Otology and X-ray-therapy. Our cordial thanks are due to Professors S.-T. Katsura, Y. Koga and T. Tsuiki for their kindness.

Miscellaneous diseases: 70 patients were taken at random. 6 cases gave a positive reaction, they are 2 cases of phthisis out of 14, 1 case of cholecystopathia out of 5, 1 stomach ptosis, 1 malnutrition and 1 erythema multiforme exsudativum.

The diseases qualified with negative reaction only are 12 ulcera (stomach and duodenum) 4 ascariasis and etc.

From the data above given, we are doubtless qualified to say, the skin injection of the watery extract of the sarcoma tissue evokes generally a certain reaction in the patients suffering from the sarcoma, while no reaction is produced in the cases with the carcinoma. The difference is decisive.

Although there were detectable exceptional cases, they were very small in number and there were apparently some grounds to account for the discordance.

The present outcome well corresponds to the finding of Kaminer[4] on the diagnosis of the carcinoma. He injected the crystallic carcinoma fatty acid intracutaneously. The reaction sets in in the carcinoma cases almost always (97%), while none in the cases with the sarcoma (4 in number).

He measured the induration, and in fact 2 days after the injection.

If both the outcomes (Kaminer and Satake) be combined, the malignant neoplasma can be diagnosed and differentiated with sure by the skin reaction against the substance which is obtainable from the neoplasma and characteristic of its properties, whether carcinoma or sarcoma. It is highly desirable to deepen this kind of researches along the process of reasoning.

Discussion.

In connection with the present communication Nakagawa's investigations[5] carried out in dealing with the same question might be referred to on this occasion. He seemed successful while the author was trying to improve the serodiagnosis of cancer, devised by Lehmann-Facius and re-

ported the availability of the sarcoma extract besides the cancer extract for discriminating them from each other.

The sera from the carcinoma patients react positively against the carcinoma extract, but does not against the sarcoma extract, but those from the sarcoma patients react equally against both kinds of extracts.

He then however suddenly withdrew his opinion in a later publications.[6] Namely he came to witness negative cases of the sarcoma reaction in a half of the sarcoma patients (4 cases).

Aoki[7] failed to duplicate the views of Nakagawa *et al.* not only rê the value of the method to differentiate between both the kinds of malign neoplasma, but also rê that of the procedure of Lehmann-Facius in general. Apparently this criticism did not remain without effect upon the above-referred withdrawal.

In his several experiments Nakagawa made extraction mostly from the sarcoma of rabbits; that of hen, rat and patient was also used. Further it should not be neglected here in referring to the outcome of Nakagawa that the cancer extract reacts invariably positive against the sarcoma sera; it is quite unintelligible from the findings here given.

Similar results to those quoted in the above paragraph were obtainable in the experiments of Matsubara,[8] who applied the same principle as Kaminer's to patients of various kinds of neoplasma. This is worth while to be discussed now.

He used the skin-test, similar to Kaminer's and according to the present worker's, but he chiefly estimated the area of redness and limited the observation within 4–1 hours after the injection. And human cancer, chorion tissue and mouse sarcoma were extracted. All the extracts caused the skin reaction in the patients suffering from all the kinds of malign neoplasma and from the pregnant women.

According to his publication in 1950,[8b)8c)] the placental extract produces the positive reaction in carcinoma patients, while none in pregnant women, if the extract is applied intracutaneously and the findings in 1–2 hours only be taken into account. Therefore the extract, prepared in the hand of Matsubara thus involves the matter characteristic to the rapidly developing cells but common to all the kinds of neoplasma, malign as well as benign. In contrast to that author both the extract of Kaminer from the cancer and that of the present writer from the sarcoma contain the matter characteristic solely to either the cancer or the sarcoma.

Summary.

From the spindle cell sarcoma originated from the great omentum in a patient, which was obtainable in a form of pure cultivation and was not contaminated with necrotic mass if any, watery extract was prepared,

and injected intracutaneously into the patients suffering from the malign neoplasma.

The cutaneous reaction was followed, and chiefly the induration estimated; its largeness at the end of 24 and 48 hours after the injection was particularly noted.

The results were clear cut, that is, commonly the sarcoma patients gives the positive reaction while the carcinoma patient does not.

The outcome thus harmonizes perfectly with that of Kaminer with the carcinoma extract. The present investigations deal with the sarcoma extract only, and Kaminer dealt with carcinoma only.

The present outcome shows, otherwise expressed, that it is possible to obtain from the sarcoma tissue the substance which brings about the skin test, described in the text, insarcoma patients only, but not in carcinoma cases. It is highly probable that our success was due to the purity of the sarcoma tissue utilized here.

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Study on the Hereditary Black Blood Disease.

(Tamura and Takahashi's Disease).

By

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In one village of Iwate Prefecture, there are many persons in specific family whose skin and visible mucous membrane appear as violet-black colour; its identity and genetic form has been not cleared until nowaday.

Since 1946, we have studied on this disease and ultimately reached the some conclusion of the reason that makes the skin of patients violet-black colour.

Why do the skin and visible mucous membrane of these patients appear as violet-black colour?

In 1937 Shikinami and Hosokawa[1] from the observation of the persons suffering from this disease, concluded that it is a hereditary Addison's disease.

This report is not right, because there is no pigment cloasma on the skin or the mucous membrane and also in the clinical symptoms as low blood pressure, muscle asthenia, and the digestive disturbances, we can not find the reason to conclude as Addison's disease.

The violet-black colour of the skin and mucous membrane is due to the blood itself, and especially Tanabe[2] in 1941 confirmed from the examination of eye ground that the artery does not appear fresh red as usual and the colour of vein is more black than the former.

By pressing the skin and mucous membrane its black colour tends to disappear and become more black in hyperemic condition of the skin by warm bathing. From these facts might be considered that it is not the pigmentation of the skin, but of this blood itself.

The serum of this black blood from elbow vein shows no abnormality and the black colour is due to the corpuscle component. The colour of stroma from the repeatedly washed erythrocytes is perfectly normal and the hemoglobin part black and when the black coloured hemoglobin is divided to the hem and globin, it is decided that the former is responsible

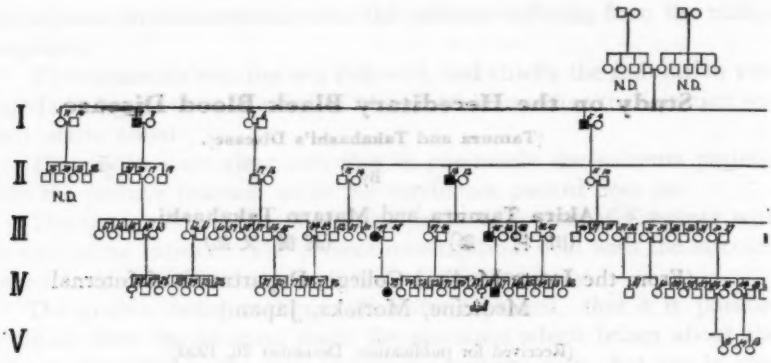


Fig. 1. Pedigree chart of the hereditary black blood disease up to IV 36 is our original patient and also the Tanabe's person. IV 31 is Shikinami's patient.

to the darkness of the colour.

Geneticobiological study of this disease.

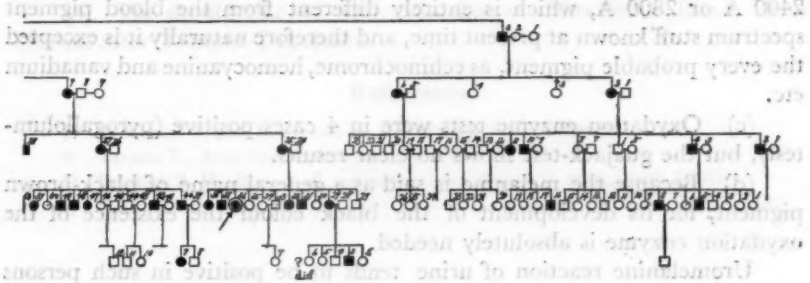
From the following pedigree chart of this family it is doubtless the hereditary disease. The sex-linked inheritance is able to be gainsaid,

TABEL I.

Generation	Healthy persons	Black-Blood disease patients
I	2	3
II	2	4
III	14	9
IV	16	17
V	5	2
Totai	39	35

TABLE II.

Generation	Brothers (suffered only)	Brothers
I	3	5
II	4	6
III	9	16
IV	16 (excluded the propositus)	32
V	2	7
Total	34	66



November 1947.
III 27 is Kimura's patient.
* d. inf. ----- dead infant.

because this one appears samely in both sex without any differentiation and it must be determined that the sickness is of dominant gene in the common chromosome, because if the parent are healthy, the children are healthy too, but if either of parents is suffering, it will be inherited. According to the observation by Weinberg's sibling method or probanden-method, it is separated to 1: 1. Therefore, it will be surely determined as a dominant hereditary disease which distributes to 1: 1. From the careful examination of the pedigree chart concerning with the theoretical and experimental calculation, it is concluded that this disease has happened by the mutation and its genetic age must be traced to 140 or 160 years ago.

Experimental study of the substance which makes the blood of this disease black.

From the well known theory that ontogenesis repeats phylogenesis we have studied along this line.

(a) According to the observation of blood specimens, we could not find any black pigment in the blood corpuscle.

As the origin of the black pigment in the hem part of blood might be considered the following pigments, for example melanine, fat, ochronose and carotin, among these pigments it comes to the question only melanine, but from our histolochemical examination of this blood, we could scarecely verified the melanine pigment.

Be that as it may the black pigment is not in granular form in the blood corpuscle, but diffusively exists in the blood cells.

And it is able to be found macroscopically when they aggregate in

group, although it is not visible in separated condition.

(b) The spectroscopic examination of this blood offers no absorptive line of methemoglobine, but shows some absorptive band between 2400 Å or 2800 Å, which is entirely different from the blood pigment spectrum stuff known at present time, and therefore naturally it is excepted the every probable pigment, as echinochrome, hemocyanine and vanadium etc.

(c) Oxydation enzyme tests were in 4 cases positive (pyrogallolum-test), but the guajack-test shows no clear results.

(d) Because the melanine is said as a general name of black-brown pigment, for its development of the black colour the existence of the oxydation enzyme is absolutely needed.

Uromelanine reaction of urine tends to be positive in such persons who are intensive of dark colour, and therefore in this case it might be considered that there is some different oxydation enzyme from normal one.

(e) We tried to reduce by putting the sulphuretted hydrogen and crystallized vitamine C into the blood of the patients; but have not found any variations of the colour.

The determination of catalase in the blood showed the normal value and too, the reduction of gluthation was within the limits of normal one.

Discriminative diagnosis of this disease.

We must differenciate this disease concerning with the black colour of skin from following condition; for example congenital heart failure, hereditary and conditional methemoglobinemia,[3] black water fever,[4] Addison's disease, scurvy etc. But it is quite easy to differenciate these diseases from this, especially in clinical symptoms and blood spectrum.

Conclusion.

(1) We have studied the curious hereditary disease which is seen in some village of Iwate prefertue in 11 families at present, and from the observation of pedigree of the families we concluded that it must have happened by mutation about 140 or 160 years ago.

(2) This disease is the Dominant Hereditary Disease which separates to 1 : 1.

(3) However the violet-black colour of the skin and mucous membrane is due to the blood itself, and except the black colour they are all in normal healthy conditions.

(4) The factor which these persons to make black is from such a substance compounded with hem, and the identity of the substance has

not been known to us until nowaday, but from the various genetical and symptomatological conditions we suppose this substance may be a sort of melanine pigment.

(5) We should like to call this disease Hereditary Black Blood Disease after Tamura-Takahashi.

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A One-dimensional Diffusion Method of Assaying Streptomycin.

(3rd Reprot)

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In a previous paper the author reported a one-dimensional diffusion method assaying streptomycin in the "blood." Contrary to this, almost all other investigators have so far been measuring the concentration in the "serum." Present paper is to discuss the difference between the "blood concentration" and the "serum concentration" and to report some improved techniques.

1) The procedure. 0.2 cc of blood was pipetted from the ear of a patient and dissolved into 1.8 cc of M/150 phosphate buffer. Thus, the hemolysis, which occured immediately, eliminated the hindernis of diffusion, which may be caused by the red blood cells. The hemolysed solutions were then superposed on about 2 cc of the germinated less nutrient agar in small test tubes. As test organisms *Sta. aureus* or *B. subtilis* were used. The standard solutions of streptomycin were made by dissolving in the same buffer and superposed.

2) Based upon the experimental data and upon the so-called hematocrit ratio, the following formula was suggested for the relation between "blood concentration" (U_B) and "serum concentration" (U_S):

$$U_s = (1.4 - 1.6) \times U_B$$

It will be seen, that streptomycin was not so much absorbed by red blood cells, but almost all the streptomycin in blood was dissolved in serum.

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Scotopic Process and On-Elements in Human Retina.

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Introduction.

It is generally believed that the scotopic process is slower than the photopic process. This view is based on the facts that the time-course of dark adaptation is slower than that of light adaptation, and that the fusion frequency of flicker is lower at the periphery than at the fovea.[1] From his studies on the time-course of electrical excitability of the eye after exposure to various colored lights, Motokawa[2] concluded that the red process is most rapid, the yellow and green processes intermediate, and the blue process slowest. The so-called rod-process which was analyzed by the same method[3] was found to be more rapid than the blue process against the supposition that the rod-process should be slower than any cone-process. The present investigation was undertaken to study more fundamentally the question as to whether or not the rod-process is really the slowest process in the human retina.

Experimental.

Method and Results.

In the present experiment the same method was used as was described in the paper cited above[2]. After a preliminary dark adaptation of about 20 minutes the electrical excitability of the eye was measured at varying moments after the end of pre-illumination. The electrical excitability was expressed in terms of a physiological quantity ζ which is defined as $\zeta = 100\,(E - E_0)/E_0$, where E and E_0 are reciprocals of electric thresholds determined with and without pre-illumination.

A circular patch of 2° in visual angle was used for pre-illumination. For further details the paper cited above should be consulted.

In the majority of previous experiments we fixed the duration of light stimuli at 2 seconds, for it was known from preliminary experiments by Motokawa that the duration of pre-illumination has no effect upon the

time-course of the electrical excitability of the eye unless the intensity of light is too weak. It has, however, been found that in the periphery of the retina and under certain experimental conditions ζ-time curves or excitability curves can depend upon the duration of pre-illumination.

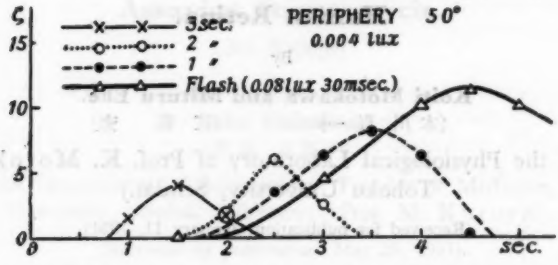

Fig. 1. Excitability curves for scotopic receptors. Ordinates: percentage increases in electrical excitability of the eye. Abscissae: time in seconds after the end of white pre-illuminating light. Duration of pre-illumination for each curve is given on top.

Such an example is shown in Fig. 1. In this experiment a retinal area 50° from the fovea was exposed to white light of 0.004 lux for varying periods such as of 0.03, 1, 2 and 3 seconds, and then changes in electrical excitability were measured in the manner stated above. Under these experimental conditions, i. e. at such a low intensity of pre-illumination and in such an extreme periphery of the retina, the photoreceptors involved must be scotopic receptors or rods; a small number of cones which might also be involved would play no important role. As can be seen in Fig. 1, the dependence on the duration of pre-illumination is such that the crest time of excitability curves as measured from the end of pre-illumination is lengthened as the duration of pre-illumination is decreased. Another aspect of dependence to be mentioned is that curves are higher with decreasing duration of pre-illumination. The crest times are about 1.5, 2.5, 3.5 and 4.5 seconds for light stimuli of 3, 2, 1 and 0.03 seconds respectively. It deseryes attention that the sum of each crest time and the corresponding duration of pre-illumination remains invariant. This means that the time interval between the beginning of pre-illumination and the crest of the excitability curve remains constant irrespective of the duration of the light stimulus. This remarkable fact has an important bearing on the type of response of the scotopic elements. This relation will, however, more fully be discussed after further details of experimental results have been presented.

On the other hand, excitability curves for cones obtained at the fovea show no dependence upon the duration of pre-illumination, as has been confirmed again in the present experiment. Fig. 2 illustrates this relation;

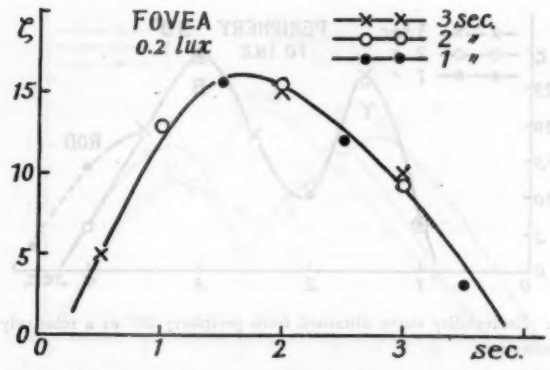

Fig. 2. Excitability curve for photopic receptors drawn with data obtained pre-illuminations of varying duration.

the data obtained with pre-illuminations of 1, 2, and 3 seconds can be represented by a single curve common to all kinds of data.

The phenomena observed above may be expressed in such a manner that the crest time of any excitability curve is independent of the duration of pre-illumination if the crest time is measured from the beginning of the light stimulus for the scotopic process, but from the end of it for the photopic process. In other words, the on-effects of the light stimulus must determine the time-course of the scotopic process, but the off-effects determine that of the photopic process.

Outside the fovea, where photopic and scotopic receptors co-exist, the crest time as measured from the end of pre-illumination either depends or does not depend upon the duration of pre-illumination according to whether the intensity of the pre-illuminating light is below or above the cone-threshold. When the intensity of light is sufficiently high, not only cones, but also rods must be excited, and one may suppose that the two kinds of receptors would equally contribute to an excitability curve determined under such conditions. As a matter of fact, however, the curve is determined by the photopic receptors alone, because ζ-values of cones are generally much higher than those of rods. An example of an excitability curve obtained at the periphery 30° from the fovea is illustrated in Fig. 3. The intensity of illumination was 10 lux, that is, much higher than the cone-threshold. The curve consists of two elevations, one at 1.5 sec. and the other at 3 sec. Based upon Motokawa's analysis[2)3)], it can be said that the elevation at 1.5 sec. represents the yellow process (Y) while that at 3 sec. represents the blue process (B). This fact corresponds to the sensory fact that yellow and blue sensations are predominant in this region of the retina. It must be noted that this curve has been

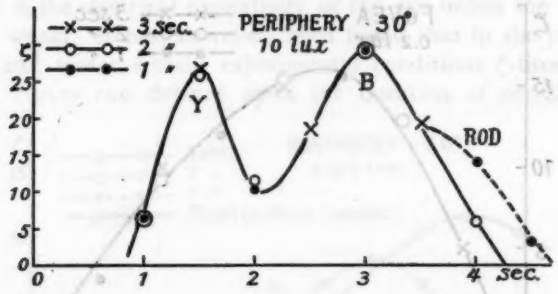

Fig. 3. Excitability curve obtained from periphery 30° at a relatively high intensity of pre-illumination.

drawn with the data obtained with pre-illuminations of varying duration, and such a consistent result provides further evidence that the photopic process, wherever it may originate, depends little on the duration of pre-illumination. It must, however, be mentioned that towards the end of the curve some discrepancy is found between the ζ-value obtained by illumination of 1 second and that obtained by illumination of 2 seconds. This difference is too great to be regarded as due to the error of measurements. The branch of the curve may, therefore, be supposed to be a manifestation of the scotopic process; as is obvious from Fig. 1, the scotopic process as determined by one second's pre-illumination has its maximum at 3.5 sec. and can survive every cone-process.

At a lower intensity of pre-illumination and in the periphery of the retina, the two kinds of process can manifest themselves in the same order of dominance, so that excitability curves obtained under such conditions can present very complicated patterns. Such cases are illustrated in Fig. 4.

The experiment was carried out at 50° from the fovea and with light stimuli of 0.2 lux lasting for 1, 2 and 3 seconds. The excitability curve for the duration of 3 seconds consists of two elevations, the one of which has a crest time of about 1.5 sec. and the other a crest time of about 3 sec. (see continuous curve). Judged from the crest times, the former elevation must represent the process Y, and the latter the process B. Thus, the photopic processes alone prevail when the duration of pre-illumination is relatively long, presumably because the scotopic curve is so low under these experimental conditions that it is covered by the process Y (cf. Fig. 1). The earlier part of the curve for the duration of 2 seconds obviously consists of the Y process, but the later part presumably belongs to the scotopic process, because its maximum lies at about 2.25 seconds in close proximity to the maximum of the scotopic process corresponding to the

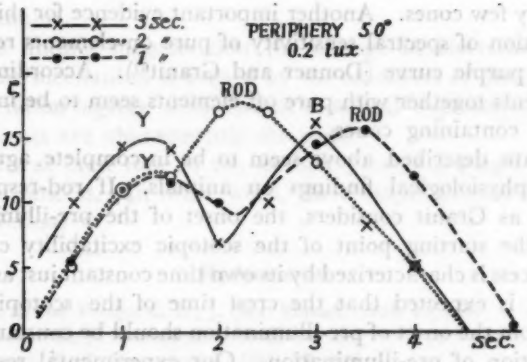

Fig. 4. Excitability curves obtained from periphery 50° at an intensity of pre-illumination low but slightly higher than cone-threshold. Full explanation in text.

pre-illumination of 2 seconds in Fig. 1. The circumstance that there should be no photopic process having such a crest time in this region of the retina is another reason for identification of this maximum with the scotopic process. The curve for the duration of 1 second consists of two elevations, the one at about 1.5 sec. and the other at 3.5 sec. The former is obviously the Y process and the latter the scotopic (cf. Fig. 1). From these examples it seems that it would be almost impossible for us to identify each elevation without the knowledge of the photopic and scotopic processes isolated in pure form.

With regard to the isolation of each retinal process the following remarks should be added. 1. For the isolation of the photopic processes it is advisable to use pre-illuminating light of sufficiently long duration so as to depress the appearance of the scotopic process. For this purpose, the duration of pre-illumination should be longer than 4 seconds. 2. For the isolation of the scotopic process it is advantageous to use a flash of light for pre-illumination, because the scotopic excitability curve is highest and longest in duration under these experimental conditions so that it survives all photopic processes. It goes without saying that the isolation becomes more perfect if the intensity of light is reduced below the cone-threshold.

Discussion.

Hartline[4] found that discharges from a single optic nerve fiber fall into three main types, namely on-elements, off-elements and on/off-elements, and this finding was confirmed by Granit with his micro-electrode technique. This author[5] considers pure on-elements to be characteristic for rods, because of their dominance in the retina of the guinea pig which

contains very few cones. Another important evidence for this view is that the distribution of spectral sensitivity of pure on-elements reproduces the pure visual purple curve (Donner and Granit[6]). According to Granit, on/off-elements together with pure off-elements seem to be in the majority in any eye containing cones.

Our data described above seem to be in complete agreement with these neurophysiological findings on animals. If rod-responses are of "on-type" as Granit considers, the onset of the pre-illuminating light should be the starting point of the scotopic excitability curve. If the scotopic process is characterized by its own time constant just as the photopic processes, it is expected that the crest time of the scotopic process as measured from the onset of pre-illumination should be constant irrespective of the duration of pre-illumination. Our experimental result is indeed just as expected from the neurophysiological finding by Granit.

Owing to our ignorance of the type of response of retinal elements we were quite at a loss when we found that the crest time of the so-called rod-curve as measured from the end of 2 seconds' illumination was shorter than that of the photopic process B, and that the crest time was found different with varying duration of the pre-illuminating light, because the former finding seemed difficult to be reconciled with the well established facts suggesting the slowness of the rod-process, and the latter appeared to contradict the law of characteristic time constants established on the cone-processes. Thanks to the significant suggestion by Granit, these questions have clearly been solved. It has been confirmed that the scotopic process is slower than any photopic process; the true crest time of the scotopic process is about 4.5 seconds while those for the red, yellow, green and blue processes are about 1, 1.5, 2 and 3 seconds respectively. Thus, our experiments have provided indirect evidence that Graint's statement applies to the human retina as well.

Summary.

Percentage increases in electrical excitability of the eye after exposure to white light are denoted by ζ.

1. At the fovea, ζ-time curves or excitability curves depend little on the duration of pre-illumination; the time interval between the end of pre-illumination and the crest of the curve or crest time reamins constant irrespective of the intensity and duration of the light stimulus.

2. On the contrary, at the periphery of the retina and at a sufficiently low intensity of pre-illumination, the crest time generally depends on the duration of pre-illumination in such a manner that it is shorter with increasing duration. The crest time remains, however, independent of the duration of pre-illumination when it is measured from the beginning of

the light stimulus.

3. This finding suggests that the change in electrical excitability in the scotopic mechanism is caused by the on-effects of illumination, and this interpretation agrees with Granit's animal experiments suggesting that on-elements are characteristic for rods.

4. The true crest time of the scotopic process is longer than that of any photopic process. In other word, the scotopic process is the slowest one in the retina.

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The Tohoku Journal of Experimental Medicine, Vol. 54, No. 3, 1951.

Studies on the Antibiotic Substances from Actinomyces.

(10th Report)

By

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(Received for publication, May 29, 1951)

A strain of Streptomyces, No. 24, which resembled *Streptomyces roseochromogenus* showed an interesting antibiotic spectrum. As usually known, streptomycin producing strains inhibit *B. anthracis* more strongly than *B. subtilis*, while strains producing streptothricin-like substances inhibit vice versa, when tested by streak plate method. The strain No. 24 which inhibits *B. anthracis* more strongly than *B. subtilis*, appeared to be a streptomycin producing strain. This property, however, was lost if it was tested by the cup assay method using its culture filtrate. These differences between antibiotic spectra has also been found in many other cases and presumed to be due to another antibiotic substance, which did not remove into the culture filtrate.

Present paper is to report on the purification of an antibiotic substance produced by the strain No. 24.

The modified Waksman's medium with 1 per cent peptone was selected as the best for shaking culture. As carbon source, glucose was found more suitable than starch or glycerol.

The method of purification was almost the same as that of streptomycin and streptothricin. By the chromatographic procedure pure HCl-salt was obtained. Its in vitro activity against almost all the pathogenic bacteria except acid-fast organisms was nearly the same as streptomycin.

Different from other streptothricin-like substances, this agent showed a good diffusibility and inhibited E. coli with a relatively small dose. The ratio of Staphylococcus unit to E. coli unit was 5:1 in this case, while for streptomycin 10:1, and for other streptothricin-like substances 100:1.

By usual procedure a crystalline helianthate (decomp. p. 280°C) and reineckate (decomp. p. 185–201°C) were obtained. Pure HCl-salt showed a positive glucosamine reaction and was also positive to Molisch's, Tollens's, indol and Fehling's reaction, while it was negative to maltol, biuret, ninhydrin, Adams-Kiewitz's, Liebermann's, Neubauer's, and Sakaguchi's tests.

Though an intramuscular injection of 20 mg of this salt was not fatal to mice, they died by an intravenous injection of 5 mg after 3 days and showed some hemorrhagic changes in the liver and lungs.

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Transorbital Brain-Ventricle Puncture or A New Method for Pneumoventriculography.

By

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(Received for publication, January 18, 1951)

Is it impossible to manipulate the ventricle puncture more easily and briefly than the routine one, or, is'nt there any way possible to take place it without making of trepan to the cranial bone? With this object, an applying of trepan by means of drill must of course be avoided. For the purpose of this matter, we have anew pursued and, at last, attempted to apply the technique for transorbital lobotomy which had already described by Freeman[1], and then, certain successes have been obtained, so that, in this paper, those results, especially those methods, are reported.

Technique and Examples.

After many trials, we have anew designed and employed the ventricle puncture needle as shown in Fig. 1, which is of course applicable as the lobotome in case of transorbital lobotomy. Owing to the bigness of the used neelde, though its stylet is the routine lobotome for transorbital lobotomy, its fixing at the orbital portion is very certain, and consequently, a manipulation of pneumoventriculography has become easily, too, since, according to the accurate fixing of the needle itself, the movements of the

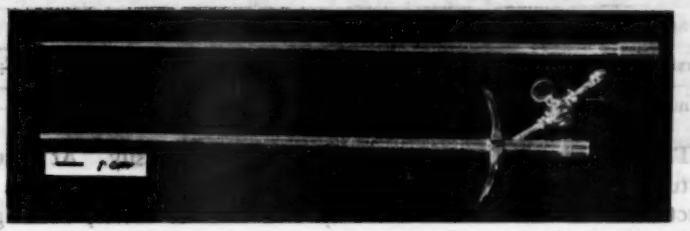

Fig. 1. The ventricle puncture needle designed by the authors. The upper: stylet; the under: tube.

neelde, or the influences due to manipulation of pneumobreathing are scarcely recognizable.

A determination of the direction of inserting needle is relatively difficult, but important. We have quantitatively measured various positions of ventricle shadows in pneumoencephalograms of the psychotics (Fig. 2 and Table I). At last, we have aimed the anterior angle of the side-ventricle as the first mark of insertion of the needle, so that its direction may be suitable, when it is inserted as the heavy lines in Fig. 2, which accord with the measured values in Table I. The distance between the orbit and the anterior angle of the side-ventricle, viz. $\overline{od}$ in Fig. 2, was ca. 8 cm.

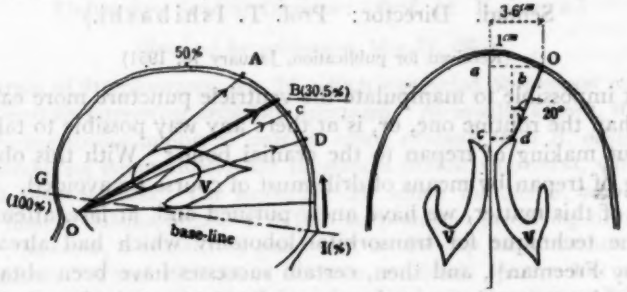

Fig. 2. Measured positions in pneumoencephalogram. G: grabella; I: inion.

Table I.

Results of Quantitative Measurement of Ventricle Shadows in Pneumoencephalograms from 15 Cases of Schizophrenia.

All marks accord with those in Fig. 2.

The angle indicates that between $\overline{OA}$ and $\overline{GI}$ (base-line), that between $\overline{OB}$ and $\overline{GI}$, ...

Value	Distance from inion				Angle against base-line (GI)			
	$\overline{IA}/\overline{IG}$	$\overline{IB}/\overline{IG}$	$\overline{IC}/\overline{IG}$	$\overline{ID}/\overline{IG}$	$\angle\overline{OA}\text{-}\overline{GI}$	$\angle\overline{OB}\text{-}\overline{GI}$	$\angle\overline{OC}\text{-}\overline{GI}$	$\angle\overline{OD}\text{-}\overline{GI}$
Meaned valve	40.5%	30.5%	27.3%	23.3%	43.1°	34.6°	30.8°	26.2°
Maximal value	+5.5"	+10.0"	+11.9"	+9.0"	+13.9°	+16.4°	+17.2°	+13.8°
Minimal value	−3.3"	−4.8"	−2.3"	−9.0"	− 7.1°	− 9.6°	− 8.8°	−11.2°

The procedure of pneumoventriculography is easily. At first, the puncture is made in a supine position. If the cerebral liquid is flew, subject's face is now turned laterally and a little lower, making the punctured ventricle downward. And then, air-liquid-exchange is taken place.

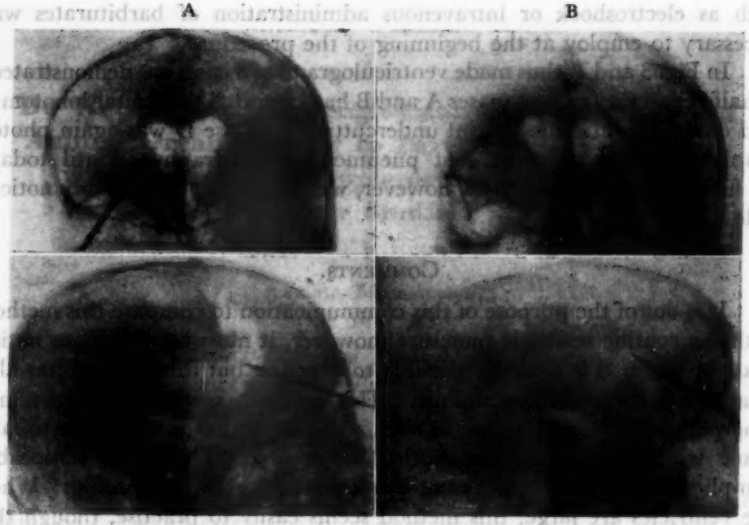

Fig. 3. Pneumoventriculograms of 2 cases of schizophrenia. Black narrow line is the shadow of inserted needle.

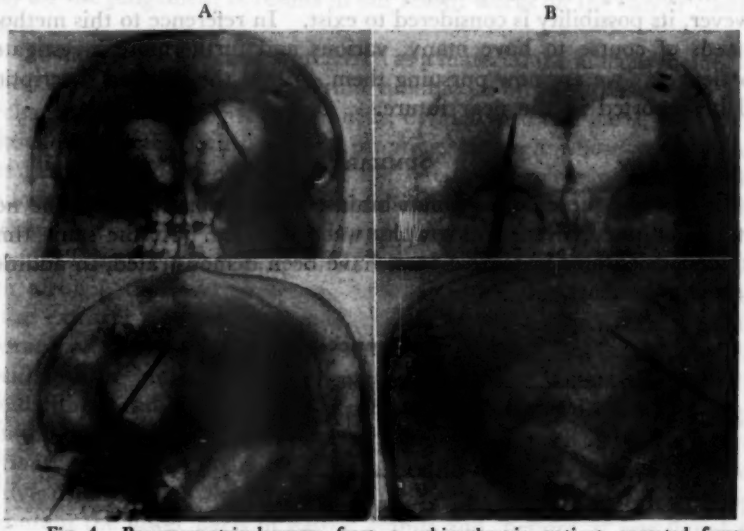

Fig. 4. Pneumoventriculograms from a schizophrenic patient operated formerly subcortical undercutting.

A manipulation of puncture needs ca. 1 or 2 min., and also, that of air-liquid-exchange, ca. 10 min., so that narcosis of shorter duration,

such as electroshock or intravenous administration of barbiturates was necessary to employ at the beginning of the procedure.

In Fig. 3 and 4, thus made ventriculograms of 3 cases are demonstrated. A half year, or more, ago, cases A and B had operated prefrontal lobotomy, and case C, frontal subcortical undercutting.[2] Case D was again photographed 7 days after the first pneumoventriculography. Until today, we have experienced 15 cases, however, we could not observed any noticeable complications.

Comments.

It is out of the purpose of this communication to compare this method with the routine ventricle puncture, however, it must be worthy to notice that this method is not only possible to practise, but this method has also established by such a technique. This method, we have thought, has long points, above all, it may be such a possiblity that it is capable to take place within short duration. Also, there are, perhaps, no noticeable complications as well as cases operated transorbital lobotomy. When the ventricles are large, this method seems easily to practise, though this item accords with those in cases of the routine ventricle puncture. We have not yet opportunities to apply this method to cases of brain-tumors, however, its possibility is considered to exist. In reference to this method, it needs of course to have many, various and furthermore investigated experiences: we are now pursuing them, so that the detailed description will be reported in the near future.

Summary.

The technique of transorbital brain-ventricle puncture and the new method for pneumoventriculography were reported. At the same time, the ventriculograms in several cases have been demonstrated, in addition to some comments.

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Do Water-extractible High-molecular Substances of Animal Organs Belong to Parenchyma or Connective Tissue? (A Supplementary Note to Our Cancer Studies.)*

By

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(Received for publication, January 22, 1951)

We have been years engaged in the study of high-molecular substances obtainable by water-extraction of animal organs. It is an essential problem if they originally belong to the parenchyma or to the stroma, particularly for the judgement of results in our recent works on the cancer components.

To answer the question I preferred as a material the mucous membrane adhered by muscularis mucosae and submucosa of pig stomach and after mechanically separating it from mucus, divided into two parts, the surface epithelium together with mucosa propriae, through whose whole thickness gastric glands penetrate, and the remaining part, by impregnating the former with a dilute NaOH and following scraping. The first batches —Tissue Portion-A's—were constituted chiefly of epithelial cells and the second—Tissue Portion-B's—chiefly of connective tissue. After boiling with alcohol, they were subjected to water extraction, and fractionation of the extracts effected by a modification of the method[1] usual in our laboratory. The yields of the fractions (Group A carbohydrate, the glucidamin or glucidamins precipitable at pH 2.0 from water and the glucidamins soluble in glacial acetic acid) from T.P.-A's were larger than those from T.P.-B's. The corresponding fractions from the two tissue portions analyzed similar. The thin lamina propria is made of connective tissue and on the other hand, T.P.-B's proved under microscope to contain the lower parts of gastric glands and, besides, it was found by a like fractionation procedure that over into the dilute alkali employed for swelling the mucosa a part of the epithelial-cellular components—

* 144th report of Masamune and co-workers' "Biochemical Studies on Carbohydrates."

Tissue Portion C's—had passed. Therefore, the relative amounts isolated of the substances from T.P.'s A's and B's are assumed as justified to indicate that, generally, water-extractible colloidal substances from organs are the components of the parenchymal cells.

Experimental.

Preliminary Separation of the Mucosa and Submucosa of Pig Stomachs.

From a pig stomach opened along the lesser curvature, the mucus was abraded and the layers mucosa and submucosa were torn off together, which were then hemmed with a thin metal wire to make a shallow cap-like bag. It was dipped in 1% NaOH as turned inside out for 30 to 90 minutes, varying according to the thickness of the mucosa (temp., 10–15°C) which thereby became swollen and translucent. After untying the wire and cutting off the margin, whose mucosa had not been soaked, the membrane was spread with the mucosa-surface upward and mucosa was scraped off—**Tissue Portion A.** The residual tissue will be referred to as **Tissue Portion B.** Under microscope (hematoxylin-eosin and van Gieson's stain) small fragments of muscularis mucosae and submucosa were seen in T.P.-A's, and those of gastric glands in T.P.-B's. The tissue portions were then dehydrated by placing in 5 volumes of alcohol for many days and after masticating, dried in a Faust apparatus. T.P.-A's changed into light lumps, brown-tinted and fragile, and T.P.-B's into white tough fibrous matter. The total amounts of the T.P.-A's and -B's separated from 1543 g. of the fresh material (from 8 stomachs) were respectively 1191 and 708 g. in a wet state and 135 and 128 g. in a dry state. The dilute lyes were colored yellowish and faintly turbid and contained some slime. Their total volume was 8920 cc.—**T. P.-C's.**

Fractionation of the Tissue Portions.

1) T.P.-A's (The epithelial cells+lamina propria).

a) Treatment with boiling alcohol. To the lumps ground were added 5 volumes of alcohol, and after acidulation with 30% acetic acid, refluxed in a water-bath for 1 hour. While hot, the alcoholic extract was separated by decantation and squeeze. (It was set aside to isolate the blood-group substance of lipoid nature. The volume was 625 cc.)[2]

b) Watery extraction and following rough fractionation. The tissue residue, dried and powdered, (134 g.) was shaken repeatedly with water (10 volumes and the decreasing) on an apparatus, lasting each time for 3 days, till an extract from which precipitation scarcely occurred on

addition of acetic acid. Room temperature, 11–17°C. Preservative, thymol. The successive extracts combined (7200 cc.; neutral, yellowish and slightly turbid) were alkalized to a pH of 9.0 by means of the saturated $Ba(OH)_2$. To the centrifuged somewhat turbid supernatant fluid were added 30% acetic acid to pH 4.5, whereupon again precipitates came out, and a little NaCl. Centrifuged after a while. The centrifugate here was washed with a dilute acetic acid at pH 4.5 and with abs. alcohol in sequence and dried in a vacuum desiccator ($CaCl_2$). A coarse powder with gray shade amounting to 0.47 g. was given. This will be referred to as **The Acetic Acid-precipitable.**

The acid supernatant mother liquid (a little turbid) and washings (yellowish transparent) were dialyzed against running water for 3 days, and after distilling in vacuo to a syrup, precipitated with about 5 volumes of abs. alcohol and some anhydrous sodium acetate. The amorphous deposits were washed with abs. alcohol and dried. The yield of a light yellow powder obtained was 4.02 g. This was taken up in 30 cc. of water and the insoluble part centrifuged and washed with water, and then the supernatant fluid and washing (about 100 cc. in total, yellowish turbid) were alkalized with drops of 20% Na_2CO_3 to a pH of 9.5, whereby turbidity increased. The centrifugate here was washed with two 20 cc. portions of a dilute Na_2CO_3 of same pH and the mother liquid and washings (135 cc. altogether) united were acidified to pH 4.0 with drops of 30% acetic acid, with following separation of the appearing abundant precipitates and washing with a dilute acetic acid (pH, 4.0) in a similar manner as above. The present mother fluid and washing (170 cc. in total) were precipitated by the aid of 5 volumes of alcohol and a little anhydr. sodium acetate. Washed with alcohol and dried.

Next, the amorphous powder obtained (2.13 g.) was made into a dough with 1 cc. of water, 60 cc. of glacial acetic acid (98%) added, in drops with kneading at first and in larger portions afterwards, (The mixture contained 96% acetic acid) and centrifuged. The supernatant fluid was light yellow and clear. The centrifugate was washed with 30 cc. of 98% acetic acid and after again centrifuging and taking up in 3 cc. of water (A homogeneous paste formed), precipitated with 42 cc. of 98% acetic acid as above (This mixture contained 90% acetic acid). The precipitates were centrifuged, washed with 90% acetic acid once and with alcohol thoroughly with centrifugation and dried—**Crude Group Carbohydrate** (1.21 g.). All of the supernatant fluids containing either 90 or 96 or nearly 98% acetic acid were precipitated by the use of 5 volumes of dry acetone (No more precipitation took place on addition of additional acetone) and from the deposits acetic acid was washed out with alcohol (A little NaCl was added when a washing changed cloudy) as completely as possible. Sub-

sequent drying gave a powder with yellow shade that weighed 0.71 g.—**Crude Fraction of Glacial Acetic Acid-soluble Glucidamins.** It contained phosphorus. Biruet and Molisch reaction were positive.

c) Purified substances.

i) *The glucidamin or glucidamins precipitable from water at pH 2.0.* The Acetic Acid-precipitable in b) (0.47 g.) was suspended in 35 cc. of water (The substance dissolved scarcely) and stirred up after addition of the saturated $Ba(OH)_2$ to pH 9.5. The mixture was stood overnight and centrifuged and the centrifugate was washed with two 20 cc. portions of a dilute $Ba(OH)_2$ of a similar pH with centrifugation. The supernatant fluids (dirty, brownish, somewhat turbid) were united and carefully adjusted to a pH of 2.0 by means of HCl (1 *N*) and the precipitates that settled out hereby were separated and washed with a dilute HCl of pH 2.0 twice and with abs. alcohol five times (In later repetitions the use of a little anhydrous sodium acetate was requisite). The dried product possessed phosphorus. Hence on it was carried out twice more the barytahydrochloric acid procedure followed each time by exhaution of glacial acetic acid-soluble contaminatns, which was exerted mostly as already described, namely, the substance obtained till then was made into a dough with 3 cc. of water and a few drops of 1 *N* NaOH (pH of the syrupy solution was 9.0), 80 cc. of 98% acetic acid added in portions and the deposits washed with 98% acetic acid and with abs. alcohol. The final product (a grayish coarse powder) amounted to 149 mg. It contained practically no phosphorus.

ii) *The blood-group carbohydrate.* The crude carbohydrate (1.21 g.) (s. b)) was dissolved in 20 cc. of water, drop by drop of 1 *N* NaOH added until no more precipitation and centrifuged (The centrifugate was little). The turbid supernatant fluid was first neutralized with 5 % trichloracetic acid and then as much solid trichloracetic acid added as to reach 5%. Voluminous deposits appeared which were centrifuged and washed with a 10 cc. and a 5 cc. portion of 5% trichloracetic acid with centrifugation. The supernatant fluids (The mother fluid and first washing were turbid) were combined and precipitated with 5 volumes of alcohol and a little anhydrous sodium acetate, washed with alcohol thoroughly and dried—0.71 g. After again treating with NaOH and CCl_3-COOH (This time centrifugation after alkalization gave an almost transparent supernatant fluid and only a trace of a centrifugate. The centrifugate after addition of solid trichloracetic acid was also little, but its supernatant fluid was quite turbid.) the substance was dissolved in 3 cc. of water, 42 cc. of 98% acetic acid added in a manner above (The concentration of acetic acid in the mixture was 90%), and shaken for 3 days on an apparatus. Centrifuged. The supernatant fluid was non-colored

and clear. The centrifugate was washed with 90% acetic acid once and with alcohol many times and dried. The processes of eliminating the contaminants insoluble in 5% trichloracetic acid (The substance was agitated with 5% trichloracetic acid and the insoluble part rejected) and those soluble in 90% acetic acid were repeated more four times, when the substance became clearly soluble in tricholracetic acid and biuret-negative. The white fine powder obtained amounted to 338 mg.

iii) *The glucidamins soluble in glacial acetic acid.* The crude fraction (0.71 g.) (s. b)) was at first freed perfectly from the glacial acetic acid-insoluble substances by repeating twice the procedure above:— It was made into a paste with a few drops of water and 45 cc. of 98% acetic acid added in portions similar to above and centrifuged. The centrifugate was washed with 10 cc. of 98% acetic acid and again centrifuged. To the supernatant fluids combined were added 3 volumes of dry ether and the deposits were washed with alcohol many times, employing a little anhydrous sodium acetate besides when an alcoholic washing was turbid, and dried. In the second similar run the part insoluble in glacial acetic acid was very small and the centrifuged supernatant fluid was perfectly clear, which was precipitated with enough acetone. Hard, light yellow grains were given.

Subsequently nucleic acids were removed as follows:— The product was taken up in 15 cc. of water (An opalescent solution formed), the saturated $Ba(OH)_2$ added in drops till a pH of 9.6 and some precipitates centrifuged off. The translucent supernatant fluid was acidified to pH 4.5 by means of 5% HCl and after centrifuging off of the precipitates (a trace) HCl was further added to the somewhat cloudy supernatant fluid till a pH of 2.0, when a large amount of precipitates appeared. The pH of the centrifuged supernatant liquid was brought back with the saturated $Ba(OH)_2$ to 4.5, some deposits were discarded and the pH was raised more as high as 9.6. Faint turbidity arose but the solution was not clarified by centrifugation. Thereupon a dilute H_2SO_4 was carefully dropped in just to precipitate all barium (The pH lowered to 1.2). And the clear mother liquid was neutralized with NaOH and distilled in vacuo to a thick solution and 5 volumes of abs. alcohol and a little anhydrous sodium acetate were added. The deposits were washed with abs. alcohol and dried. However, at this stage, phosphorus was found still remaining, though little. By repetition twice more of the whole procedure, its amount could be reduced to negligible. Finally to diminish ash, electrodialysis (a Pauli apparatus, 200 volts) of the product in 70 cc. of water was conducted against changes of water after prior usual dialysis (Almost no precipitates came out by the electrodialysis), then the solution was concentrated to a small volume and the flocculent precipitates by 5 volumes

of abs. alcohol (A little anhydrous sodium acetate was used together) were well washed with abs. alcohol and dried. 52 mg. of a light brown, coarse powder were given. P —.

2) T.P.-B's (Chiefly composed of submucosa and muscularis mucosae).

T. P.-B's dehydrated (128 g. altogether) were at first treated with boiling alcohol as T. P.-A's. In this case 5 and 3 volumes of alcohol were used in succession. The durations of reflux were 1 and 2/3 hour respectively. The extracts were combined (930 cc.) and set aside.[2)]

Extraction with water of the tissue residue (117 g.) and following fractionation was carried out in a quite similar manner as above and 0.57 g. of The Acetic Acid-precipitable, 0.36 g. of Crude Group Carbohydrate and 0.39 g. of Crude Fraction of Glacial Acetic Acid-soluble Glucidamins were given. The crops of the glucidamin or glucidamins precipitable from water at pH 2.0 and of the purified products from the last two crude substances were respectively 106, 167 and 34 mg.

3) T. P.-C's (The alkali used for swelling the mucosa).

They were acidulated (to pH 5.5) with conc. acetic acid whereupon voluminous precipitation occurred. The precipitates were centrifuged, washed with abs. alcohol and dried—**Acid-insoluble I's** (hard, fragile, dark brown clumps; 5.98 g.). The yellowish nearly clear mother liquids were dialyzed against tap water for 3 days and after centrifuging off some deposits (The supernatant liquid was transparent) distilled under diminished pressure to about 10 cc. and 50 cc. of abs. alcohol and a little anhydrous sodium acetate added. The precipitates here, washed with abs. alcohol and dried, (a dirty brown powder; 7.64 g.) were taken up in 80 cc. of water, 10% HCl added until the pH reached 2.0 (Acidification with acetic acid to pH 4.5 gave a turbid centrifuged supernatant liquid) and centrifuged to separate the voluminous deposits, which were also washed with abs. alcohol exhaustively (A little anhydr. sodium acetate was used when necessary) and dried. The product (a coarse grayish powder weighing 2.95 g.)—**Acid-insoluble II's**—was put together with Acid-insoluble I's for the preparation of the glucidamin or glucidamins that are insoluble at pH 2.0. The united material does not correspond accurately to those named The Acetic Acid-precipitable, because the lyes had not been treated with baryta in advance to acidification and, on the other hand, the acid-insoluble part of the alcoholic precipitates above was incorporated on the assumption that alkali had possibly extracted substances from the mucosa which hinder the precipitation of The Acetic Acid-precipitable in the lyes. Nevertheless, the purified product

(761 mg.) from the present material showed analyses resembling the corresponding products from the other T.P.'s (s. below). Fractionation was done as in 1) and 2).

The clear supernatant fluid of Acid-insoluble II's was dialyzed and after concentration, precipitated as above. 1.70 g. grayish substance was given. From this, Crude Group Carbohydrate and Crude Fraction of Glacial Acetic Acid-soluble Glucidamins were separated by following the procedure in 1) b). The respective yields were 0.63 and 0.35 g. And 100 mg. of the blood-group carbohydrate as a white powder (biuret—) and 24 mg. of the purified glucidamins soluble in glacial acetic acid (P—) were obtained.

The final crops will be listed because of expedience.

	T.P.-A's (Chiefly epithelial cells) (135 g.)	T.P.-B's (Chiefly submucosa) (128 g.)	T.P.-C's (Alkaline extracts) (8.92 *l*)
The glucidamin or glucidamins prec. at pH 2.0 (mg.)	149	106	761
Group carbohydrate (mg.)	338	167	100
The glacial acetic acid-sol. glucidamins (mg.)	52	34	24

Composition and Other Properties of the Purified Products.

Irrespective of their source the corresponding products possessed similar appearance and properties.

The precipitable at pH 2.0 were practically free from phosphorus. Molisch and biuret, positive. They were insoluble in water but gave a viscid solution in dilute alkali.

The group carbohydrate preparations were not perfectly soluble in water, but on addition of a dilute alkali a clear solution (not viscous) formed at pH 9.0. Phosphorus was not detected. Biuret negative. Molisch positive.

The fractions composed of glucidamins soluble in glacial acetic acid showed extreme solubility, giving a clear non-viscid solution, which was partially precipitated by trichloracetic acid. Free from phosphorus. Biuret, Molisch and Osaki-Turumi (the direct as well as the indirect), all positive.

By the techniques often employed in our laboratory were obtained figures in Tables I–III.

Shortage of material did not allow the measurement of $[\alpha]_D$ and anti-isoagglutinative force of the products soluble in glacial acetic acid from T.P.'s B's and C's. The optical rotations of the substances precipitated at pH 2.0 were not accurately determined because of the opalescence of

TABLE I.

Composition and Anti-isoagglutinative Force of Fractions Containing a Glucidamin or Glucidamins Precipitable at pH 2.0.

The compositions are expressed as percentages and the anti-isoagglutinative forces in maximum dilutions of the substances, where the isoagglutination of Group A blood cells was entirely inhibited.

Analysis	The substance from		
	T.P.-A's (Chiefly epithelial cells)	T.P.-B's (Chiefly submucosa)	T.P.-C's (Alkaline extracts)
Nitrogen	13.6	13.4	14.0
Hexosamine	4.7	5.6	5.0
Non-amino sugars as galactose			
By the orcinol r.	9.8	8.2	8.0
By the indole r.	10.1	11.2	10.6
By the diphenylamine r. I	12.1	10.8	11.2
Ash	12.1	12.1	8.8
Anti-isoagglutinative force	$1:10^5$	$1:10^5$	$1:2\times10^5$

TABLE II.

Composition, Optical Rotation and Anti-isoagglutinative Force of the Blood-Group Carbohydrate Preparations from the Three Tissue Portions.

The compositions and anti-isoagglutinative forces are expressed similar to those in Table I.

Analysis	The substance from		
	T.P-A's (Chiefly epithelial cells)	T.P.-B's (Chiefly submucosa)	T.P.-C's (Alkaline extracts)
Nitrogen	4.2	4.1	4.8
Hexosamine	39.5	39.4	40.6
Non-amino sugars as galactose			
By the orcinol r.	42.1	40.7	42.2
By the indole r.	40.3	40.9	39.1
By the diphenylamine r. I	37.9	38.9	41.1
Ash	8.5	11.4	5.8
$[\alpha]_D$ (in water) on ash-free basis*	−2.9°(19.5°C)	−4.1°(19°C)	−3.7°(17°C)
Anti-isoagglutinative force	$1:8\times10^5$	$1:10^5$	$1:2\times10^5$

* The substances were dissolved by means of NaOH. The pH of the solutions was 8.0–9.0.

their solutions (prepared by the aid of alkali).

From the nearly coinciding analyses and other properties, the corresponding products from the different tissue portions are regarded as justified

TABLE III.

Composition, Optical Rotation and Anti-isoagglutinative Force of the Fractions Containing Glucidamins Soluble in Glacial Acetic Acid from the Three Tissue Portions.

Expressed like as in Tables I and II.

	The substance from		
	T.P.-A's (Chiefly epithelial cells)	T.P.-B's (Chiefly submucosa)	T.P.-C's (Alkaline extracts)
Nitrogen	10.4	10.5	9.8
Hexosamine	13.9	13.9	14.0
Non-amino sugars as galactose			
By the orcinol r.	17.4	19.1	21.0
By the indole r.	23.3	24.1	23.6
By the diphenylamine r. I	25.3	27.5	28.4
Ash	13.9	11.5	10.3
$[\alpha]_D$ (in water) on ash-free basis	−48.3°(12.8°C)		
Anti-isoagglutinative force	$1:10^3-2\times10^3$		

to be identical with one another, if allowance is made for the difficulty in separating such high-molecular substances from animal tissue and also for the fact that they (the carbohydrate excluded) are apparently mixtures.

SUMMARY.

From pig stomachs mucosa and submucosa were mechanically separated together and then roughly divided into themselves after swelling the mucosa by the aid of a dilute NaOH. The dilute alkali, which had drawn out the components of the epithelial cells in part, and the watery extracts of the mucosa and of sumbucosa were fractioned to obtain from each 1) a fraction containing a glucidamin or glucidamins precipitable from water at pH 2.0, 2) that containing glucidamins soluble in glacial acetic acid and 3) the group carbohydrate. The corresponding products from the different sources proved similar in composition and other respects (optical rotation, anti-isoagglutinative force and else). The largest yields of them were given from the mucosa except that, among the fractions precipitable at pH 2.0, the one that had been extracted by the alkali exceeded markedly the one from the mucosa (The crude material from the alkali did not exactly correspond to those from mucosa and submucosa).

The finding probably leads to a general conclusion that the water-extractible high-molecular components of animal organs belong to parenchymal cells.

Through the Grant Committee for Scientific Rresearches was given a grant from the Education Department in aid to us. H. Masamune.

References and Notes.

1) Cf. f. i. Satoh (Tohoku J. Exp. Med., 1949, **51**, 275) and Oh-Uti (Ibid., 1949, **51**, 297).

2) The experiments regarding this extract will be accounted for elsewhere.

The Tohoku Journal of Experimental Medicine, Vol. 54, No. 3, 1951.

Dorsomedial Thalamotomy (III): Influence upon Function of Autonomic Nervous System.

By

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(Received for publication, Febuary 22, 1951)

Since autumn, 1949, we have practised "dorsomedial thalamotomy," a sort of surgical invasion to the dorsomedial thalamic part, which had already been described by Wada.[1] Now, in order to investigate the influence of this operation upon the function of the autonomic nervous system, this experiment has been undertaken in 10 cases of various psychoses.

The examination, viz. adrenalin-, atropin- and pilocarpin-tests, was carried out by means of the routine laboratory examining method in every other day, 2 weeks before and after performance of the operation and the results were as follows:

Atropin-test

Case	Blood press.	Pulsation	Inc. of reflex	Thirstiness	Mydriasis	Palpitation
I	−(−)	−(−)	−(−)	+(+)	−(−)	−(−)
*II	−(−)	−(−)	+(−)	±(−)	−(−)	−(−)
III	−(−)	−(−)	−(−)	−(−)	−(−)	−(−)
IV	−(−)	+(+)	++(+)	−(−)	−(−)	−(−)

Adrenalin-test

Case	Blood press.	Pulsation	Inc. of reflex	Tremor	Mydriasis	Palpitation	Face paleness
I	+(−)	+(−)	++(−)	++(−)	−(−)	+(−)	−(−)
*II	+(+)	−(−)	±(−)	+(−)	−(−)	+(±)	−(−)
III	−(−)	±(−)	−(−)	−(−)	−(−)	−(−)	−(−)
IV	+(+)	−(−)	++(−)	+(−)	−(−)	+(+)	+(−)

Pilocarpin-test

Case	Blood press.	Pulsation	Inc. of reflex	Salivation	Sweating	Epiphora	Faceredness
I	−(−)	−(−)	±(±)	++(++)	+(+)	−(+)	+(+)
*II	−(−)	−(−)	±(±)	−(−)	+(+)	+(+)	+(+)
III	−(−)	+(−)	−(−)	++(++)	+(+)	−(−)	+(−)

Signs in parentheses indicate the data following operation. * The case which has been manipulated thalamotomy ca. 3 months after performance of prefrontal lobotomy. Case I, II & III are schizophrenia and Case IV, epilepsia (essential).

In this table, the data from 4 cases were picked up, though the other cases showed the almost identical tendency, as well as intensity, with those of Case I. It may be said, at least, that there did not occur any significant abnormal reactions and remarkable changes in intensity, rather reversely, the intensity of reaction tended to decrease, though in the adrenalin-test, a little remarkable change was observed in comparing with the two other tests. In other words, those findings might indicate that the lability in function of the A.N.S., or the tendency toward vagotony, both mentioned by many authors[2] as the occasional characteristics of the psychoses, tends to decrease by the operation.

Conclusion.

1) The influence of the dorsomedial thalamotomy operation upon the function of the autonomic nervous system was found insignificant and 2) the function of the autonomic nervous system, or its reaction, seemed to tend to be stable after the operation.

References.

1) Wada, Folia Psychiat. Neurol. Japon., 1951, 4, 309,
2) Frank, Bumke's Handb. d. Neurologie, 1936, IV, 1023.

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A Modification of Sato and Sekiya's Copper Peroxidase Reaction.

By

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(Received for publication, March 19, 1951)

There are a number of peroxidase methods for blood smears. Sato and Sekiya's peroxidase reaction[1] is regarded as one of the most recommendable methods. The Tohoku Pediatric Method[2] of staining blood films for daily routine work, published in 1934, is one of the modifications of the above peroxidase reaction.

Now I have devised another modification of Sato and Sekiya's original method. In this modification the stain time is made shorter, though the preparation of the Solutions required is somewhat more complicated.

Solutions Required.

Solution I. Iodine-iodate.Copper Sulphate Solution. To 100 c. c. of aqueous 10% $CuSO_4 5H_2O$add (see below) 5 c. c. of the following solution.

Iodine..	1.0
Potassium iodiate ..	2.0
Distilled water ad ..	300 c.c.

Adding of 5 c. c. of this Iodine-Iodate Solution to the copper sulphate solution should be performed very slowly.

Solution II. This is prepared by mixing Solutions II A and B.

Solution II A. Safranin-Malachite green Solution.

To 100 c. c. of aqueous 1% safranine solution add 40 c.c. of aqueous 1 % malachite green solution and shake thoroughly, so that a good mixture may ensue. This mixture should be left standing for one month, at least for one week, so that a uniform staining of a blood film may result.

Solution II B. Benzidine-peroxidase Solution. This is prepared in just the same way as Solution II of Sato and Sekiya's original peroxidase method. viz. rub 0.2 grms, benzidine base (this should be a good specimen) in a mortar with a few drops of distilled water. Then make the whole amount up to 100 c.c. with distilled water. Add 2 drops of (fresh) 3% H_2O_2 Solution.

Solution II is prepared by mixing Solutions II A and B together in equal parts. The solution will keep as long as 2 months.

Read at the 2nd Meeting of the Tohoku District Society of Pediatrics and of Hematology held 1 October, 1950, at Morioka City.

Technique.

Stain blood smears within 48 hours of obtaining.

Use blood smears within 48 hours of obtaining.

Cover air-dried blood smears (cover slides preferably) with Solution I and pour off most of it after 4–5 seconds. Pour ample amount of Solution II without rinsing (of Solution I) and keep staining for 1½–2½ minutes. This fluctuation of time depends on how long ago Solution II was prepared. With a freshly prepared solution 1½ minutes will be long enough, while with the oldest available solution (that is, prepared 2 months ago) 2½ minutes will be necessary.

Stained films should be made and kept dry, or else malachite green would fade away rapidly so as the whole picture would become indistinct. Well stained specimens may be kept for demonstration as long as one month if kept dry enough.

Examine the stained smear by use of cedar oil, stained side of the slide upwards.

Macroscopic Appearance of Blood Films Stained.

The slides statined with the original Sato and Sekiya method appear macroscopically faintly reddish, whereas those stained with the present method of mine appear macroscopically faintly bluish.

Microscopic View.

Leucocytes are generally stained purplish. Peroxidase-positive granules in the cytoplasma are stained greenish in contrast to the blue colour as in the original method. The greatest difference in stain result between this and the present method is the total absence of needle like protrusions of eosinophils in the present method. Besides, the peroxidase granules in this kind of cells look, I believe, larger than in the original method.

The Present method has, I believe, the following 6 merits:—

1. Stain time is shorter.
2. Stain technique is easier.
3. The nuclei of neutrophils are especially distinct.
4. Eosinophils are especially distinct.
5. Visual fields present bluish tint, agreeable to the eye.
6. The reagents will keep as long as 2 months, though the preparation is more complicated.

Summary.

I devised a modification of Sato and Sekiya's peroxidase reaction. Though the preparation of the required solutions is rather more complicated than those for the original method just mentioned, yet the time required for staining is shorter and leucocytes are more dinstinctly stained. Thus the method is, I believe, recommendable for practical hematological work.

References.

1) Sato, A. and Sekiya, S., Tohoku J. Exp. Med., 1926, **7**, 111; Naegeli, Blutkrankheiten und Blutdiagnostik, Berlin, 5th Ed., 1931, 81; Shleip and Alder, Atlas der Blutkrankheiten, Berlin & Wien, 3rd Ed., 1939, 38; Kracke and Garrer, Diseases of the Blood and Atlas of Hematology, Philadelphia, London & Montreal, 2nd Ed., 1937, 308 & 478: Wintrobe, Clinical Hematology, Philadelphia, 2nd Ed., 1947, 280.

2) Sato, A. and Suzuki, T., ibid., 1936, **26**, 93.

The Tohoku Journal of Experimental Medicine, Vol. 54, No. 2, 1951.

Studies on the Antibiotic Substances from Actinomyces.

(11th Report)

By

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(Received for publication, May 29, 1951)

The antibiotics obtained from actinomyces, such as streptomycin, chloromycetin, and aureomycin, seem to be very benficial against typhoid infections.

During the systematic study on the antagonistic actinomyces in our laboratory, streptomycin and many streptothricin-like substances were isolated and crystallized. The present study deals with the activities of streptomycin and two streptothricin-like substances against Eberthella typhosa both in vitro and in experimental infection.

Materials: 1) Streptomycin used was HCl-calcium chloride double salt (Merck).

2) Roseomycin (No. 36 HCl-salt) was a representative substance of the so-called streptothricin type 1 (ST 1) of our laboratory, which was characterized in low toxicity, good diffusibility and low potency against gram positive organisms.

3) No. 259 HCl-salt was a representative substance of the so-called streptothricin type 2 (ST2), and characterized in high potency, less diffusibility and relatively high toxicity.

Experimental infections were made in mice weighing 15–23 g with E. typhosa (T-63 strain), suspended in 5 per cent mucin solution.

Results:1) The activity ratio of streptomycin, roseomycin and No. 259 substance against E. typhosa T-63 strain tested by the dilution method was 1:1:10. In this respect, No. 259 substance was most effective. But when the activity was tested by the pulp disc method, both streptomycin and roseomycin showed higher potencies than No. 259 substance.

2) All these antibiotics effectively protected the mice in almost same doses. Therefore, No. 259 substance did not show remarkable results in proportion with its dilution unit.

3) From the facts mentioned above it was suggested that the effectiveness of drugs in vivo may be somewhat closely related to the diffusibility in vitro.

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Dorsomedial Thalamotomy (IV): Somatic Side-effects following Operation.

By

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(Received for publication, March 19, 1951)

Although the description on the complications of dorsomedial thalamotomy,[3] or a surgical invasion to the dorsomedial part of thalamus, is the purpose of this report, it would be interesting to note the clinical relationships between thalamotomy and thalamic syndrome.[1] In other hand, if there were introduced severe complications by operation, it is obvious that thalamotomy is inapplicable for clinical usage. Certain answers to thses problems are made as conclusion in this paper, basing upon the clinical observations, from one month to a half year after operation, of the subjects consisting of 41 cases of various psychoses.

Clinical Findings.

Among 41 cases, one case had died owing to the haemorrhage of hypothalamic part, on which an autoptic photograph is shown in Fig. 1. In 5 cases which were thalamotized alone, and 2 cases, thalamotized after performance of prefrontal lobotomy operation undertaken 3, or more, months ago, there occurred such disturbances that might be possible to say as hemiplegia, accompanying weakness of motility and hypesthesia of the unilateral extremities. Among them, each of 3 cases showed remarkable foot clonus and Babinski'reflex, and also, slight distrubance of light reflex of pupil. Those hemiplegia and symptoms of pyramidal system are seemingly introduced by the impairment, perhaps the lesions of internal capsule, due to technical failure; those patients showed psychic excitation during operation and, accordingly, the position of inserted thalamotome shifted in accordance with an abrupt, active movement of the head. However, those symptoms of pyramidal system had in each case reached till an almost complete recovery with gradual improvement within a half year.

In the remaining 33 cases, in which such 9 cases that had been lobotomized in former days, are included, the main side-effects recognizable

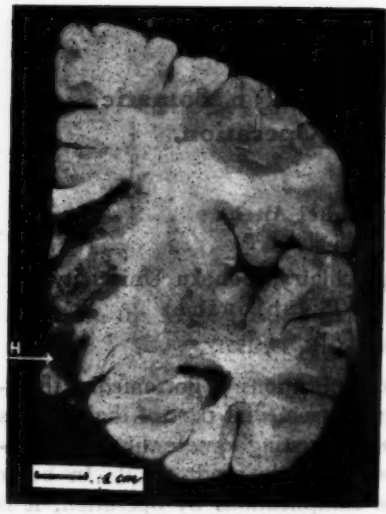

Fig. 1. Frontal section of the brain in one case of genuine epilepsy, which had died owing to the haemorrhage (H) of hypothalamic part following thalamotomy operation.

after operation are as shown in the following table.

Immediately after operation, there occurred such a disturbance of consciousness that continued for one or 2 days, however its rate and intensity were insignificant, when compared with that usually observable in the lobotomized cases. In several cases, disorientation was recognized, though it was generally incomplete. A tendency toward urinary incontinence continuing for one or 2 days was also observed, and in one case which was a deteriorated schizophrenia, this disturbance had continued for almost one month, though inconstantly. In function of reflex, a tendency toward increasing, even accompanying occasionally a transient appearance of pathologic reflexes, was dominant, but such symptoms did

Symptom which was recognized	Within one week after operation	20 days after operation
Disturb. of conscious.	13 (5)	0 (0)
Urinary incontinence	5 (6)	0 (1)
Reflex increasing	13 (5)	0 (0)
Reflex decreasing	3 (0)	0 (0)
Headache	24 (9)	0 (0)
Xanthochromia	20 (9)	0 (0)
Ataxia (static)	17 (7)	2 (2)

Sygns in parenthese indicate the data from 9 cases which were lobotomized in former days, such as 3, or more, months before application of thalamotomy.

not continue for more than 10 days, especially pathologic reflexes were temporary.

Almost all cases were used to complain of severe headache for one week after operation, or until discharge of cerebrospinal liquid was performed: in fact, this symptom was used to disappear immediately after performance of lumbal puncture. The liquid discharged in the first one week after operation was in almost all cases xanthochromic, occasionally haemorrhagic. Accordingly, an increasing of liquid pressure, perhaps

due to haemorrhage within the cerebral ventricles owing to surgical manipulation, might be considerable as one of genesis of occurrence of such a symptom. Differing from this headache, there was another headache which occurred in almost all cases during operation. It was a severe, intolerable pain without exact localization, continuing only during incisioning manipulation by means of thalamotome. Though this pain of the head accompanies with sweating and redishness of the face, it is seemingly a certain central pain having some accordances with the so-called thalamic pain.

An occurrence of ataxia, though it was in the main static one, was relatively dominant. However, this symptom tended also reduce with gradual course and extinguished till one month after operation as shown in Fig. 2. Disturbances of sensation were generally insignificant. Owing to disturbance of consciousness, its exact recognition in the early stage after operation was difficult, though its occurrence seemed to be considerable to exist. Hypersthesia or hyperalgesia did not occur, but in one case, a complain of paresthesia, such as paralgesia of the unilateral arm, was recognized for several days following operation. In the cases having ataxia, hypesthesia accompanied with sensation of weakness of motility of the extremities was observed from one to 2 weeks. At the same time, such subjects were in general used to complain of drop of grip strength, however its measurment by means of dynamometer did not show so remarkable decrease. By this reason, in cases having such symptoms, certain concernings with the disturbance in sphere of deep sensation, rather than simple alternation of superficial sensation, seemed to be impossible to deny, however an exact existence of disturbance of deep sensation was somewhat obscure. Such disturbances of sensation were transient and also used to extinguish prior to disappearance of ataxia, excepting of cases having hemiplegia. In addition, we have not experienced yet an occurrence of epileptic seizures due to thalamotomy.

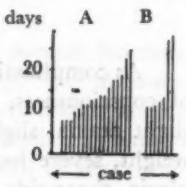

Fig. 2. Vicissitude of ataxia following thalamotomy operation. A:the cases manipulated thalamotomy alone: B: the cases received thalamotomy after application of prefrontal lobotomy made in former days.

Discussion.

In comparison with the report made by Spiegel et al[3] on somatic side-effects following thalamotomy operation, there exists a great deal of resemblances[4] and lacks of any significant differences, excepting of occurrence of ataxia and headache.

In general, complication following thalamotomy operation might

be said as slight, since it was transient and tended to extinguish. And, the occurred symptoms seemed somewhat differ from the thalamic syndrome. For example, there, according to our findings, lacked of occurrence of abnormal movement of the body, or of appearance of taxia with astereognosis or loss of sensation. In conclusion, these matters, or the above described findings, might admit to say that there exists a possibility of thalamotomy in clinical usage.

It was impossible to deny that there occurred a certain state of inertia following operation, since decreasing of appetite and weight was in all cases observed, in spite of that an increasing of temperature above 38.5° C was not recognized. We have thought, accordingly, that the complete recovery of the somatic condition from influences of operation might need at longest one month after operation.

Summary.

As complication of dorsomedial thalamotomy, hemiplegia, disturbance of consciousness, urinary incontinence, abnormality of reflex function, slight ataxia, slight disturbance of sensation, decreasing of appetite and weight, severe headache, etc. were observed, however excepting of hemiplegia, those side-effects seemed to be transient and to tend to extinguish within about a half month after operation. In adding to an existence of possibility of thalamotomy for clinical practice, that complete recovery of somatic condition might need at longest one month after operation, was concluded.

References and Notes.

1) Langworthy and Fox, Arch. int. Med., 1937 **60**, 203.
2) Spiegel, Wycis and Freed, New York State J. Med., 1949, **49**, 2273.
3) Wada, Folia Psychiat. Neurol. Japon., 1951, **4**, 309.
4) This item may indicate that the principles on thalamotomy operation might eventually same, though the techniques for operation differ between Spiegel and his collaborators and the authors.

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Dorsomedial Thalamotomy (V): Results of Psychological Examination.

By

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(Received for publication, March 19, 1951)

For the purpose of investigation of the changes in mental function, which might be introduced by dorsomedial thalamotomy,[4] the measurements by means of various psychological tests were undertaken, and in this report, the results from the following tests are described: 1) Awaji's test of version,[1] a sort of test applicable for measurement of tendency[6] of character or temperament; 2) Miyake's test of impressibility,[2] a sort of test of attention; 3) Miyake's test of association;[7] 4) Noken's test of intelligence,[3] a performance-test, or a beta-test (the full point is 50) and 5) Kraepelin's test (successive adding test).[5] Each test was taken place in every day within 2 weeks before operation and in the first 3 and 4 weeks after operation.

Fig. 1 and Table I represent summarily the results from 19 cases

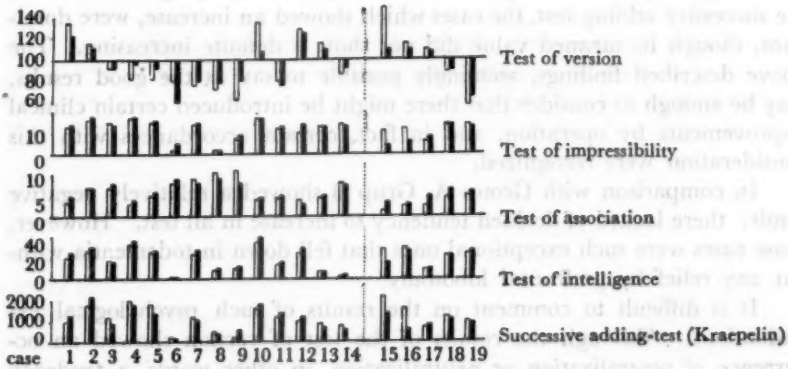

Fig. 1. Results of psychological measurements in nineteen thalamotized cases. (Black squares are the postoperative data)

TABLE I.

Results of Quantitative Analysis of Data shown in Fig. 1.

"Maximum value" indicates the data in cases which showed the maximum value among all cases, and signs in parentheses, the data in Group B.

Sort of test	Increasing		Decreasing		Average val. in all cases (in %)
	Number of case	Maximum value (%)	Number of case	Maximum value (%)	
Version	6 (2)	+ 21 (+ 14)	7 (3)	−25 (−26)	− 3 (− 8)
Impressibility	9 (1)	+150 (+ 33)	2 (3)	− 7 (−63)	+ 5 (−12)
Association	8 (5)	+ 47 (+112)	5 (0)	−47 (−13)	± 0 (−50)
Intelligence	11 (2)	+ 33 (+ 11)	1 (2)	− 5 (−44)	+11 (−12)
Successive adding	6 (1)	+140 (+ 12)	8 (4)	−25 (−57)	± 0 (−25)

consisted of Group A which was thalamotized alone, and Group B, thalamotized after prefrontal lobotomy operation made 3 or more months ago.

At first the description on Group A is made. In the test of version, the results are generally uniform: in addition to a small drop of the averages, the distribution of the points obtained seems to contract. This item, viz. centralization or approach toward 100, may indicate that the emotional condition of the operated subjects tends to be more neutral and stable, since the tendency to decline to the remarkable negative or positive parts become to extinguish. The results of the test of impressibility showed a weak increasing, viz. a slight tendency of increasing of ability for attention. In case of the test of association, none of definite tendency or change was recognized. The results from the test of intelligence showed a small increasing, and this item opposites those reported by Spiegel et al.[5] In the successive adding test, the cases which showed an increase, were dominant, though its meaned value did not show a definite increasing. The above described findings, seemingly possible to say as the good results, may be enough to consider that there might be introduced certain clinical improvements by operation, and in fact, certain accordances with this consideration were recognized.

In comparison with Group A, Grup B showed a relatively negative result: there lacked of decided tendency to increase in all test. However, those cases were such exceptional ones that fell down in todementia without any relief by prefrontal lobotomy.

It is difficult to comment on the results of such psychological examinations. Although the results of the test of version showed an occurrence of centralization or neutralization, in other words, a tendency toward stabilization, the thalamotized cases seem, even from clinical

standpoints of view, to obtain a more remarkable stability in emotional pattern, than that in the lobotomized cases. In fact, psychomotor excitation, confusion, blocking of thought, hypertension, etc. tended to disappear, none the less, disturbance of attention or will, such as indifference, lacking of spontaneity, etc. did not follow. We, therefore, have thought that the good results in psychological examinations might mainly due to the improvement in sphere of emotional pattern.

Conclusion.

The relatively good results from the measurements by means of psychological tests of version, impressibility, association, intelligence and capacity for mental work (Kraepelin's test) were recognized after performance of dorsomedial thalamotomy, though such results seem being introduced, above all, by an improvement of sphere of emotional pattern.

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6) In this test, the scale is as follow: the case with points below 100 tends to introversion and that with points above 100, to extroversion, since the case with points 100 indicates neutral.
7) In this test, the association-time was mainly pursued and, accordingly, the data shown an increase mean the prolongation in responsibility.
8) The data resulting from the numbers calculated during an hour were presented.

The Tohoku Journal of Experimental Medicine, Vol. 54, No. 3, 1951.

Studies on the Antibiotic Substances from Actinomyces.

(12th Report)

By

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In this laboratory many streptothricin-like basic substances, effective against a number of gram-negative organisms, were isolated, so the present studies were undertaken to examine the activities of these substances against *H. pertussis* in vitro and against experimental murine pertussis, being compared with those of streptomycin and " colistin " which was supplied from Kobayashi Pharmaceutical Co., Ltd. " Colistin " is an aerosporin-like substance produced by a spore-forming bacteria and purified by the same method with that of aerosporin.

Materials: As basic substances from Actinomyces, Nos. 36, 20, 259 substances and " Seki " substance were used. The former two belong to so-called streptothricin type 1 (ST1), characteristic in their low toxicity, in relatively low inhibitory action and in good diffusibility, while the latter two belong to so-called streptothricin type 2 (ST2), characteristic in their high inhibitory action, in relatively high toxicity and in less diffusibility. All these substances were used as HCl-salt.

Results: 1) Against 6 strains of *H. pertussis*, these substances were effective in vitro in the following order; colistin, streptomycin, " Seki " substance, No. 259, No. 36 and No. 20 substances. The susceptibility of *H. pertussis* to streptomycin and streptothricin-like substances was nearly the same with that of *E. coli*, while the former organism were more susceptible to colistin than *E. coli*.

2) For the experimental infection in mice, both intranasal and intracerebral inoculation methods were used. The former method needed relatively thick suspension of organisms, in order to kill all control animals within 5 days, while the latter method required a relatively small number of organisms, to cause the death of animals within 9 days. Using both these methods it was clearly shown that streptomycin and " Colistin " difinitely protect against the experimental infection.

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Dorsomedial Thalamotomy (VI): Influence upon Blood Pressure.

By

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Some experimental reports on the existence of certain relations between blood pressure and frontal lobe, or lobtomy, have been described by some investigators.[1)2)] Then how are the influence of dorsomedial thalamotomy[3)] upon blood pressure? For the purpose of explanation of this matter, blood pressure in many cases consisting of various psychoses was measured before operation and in the first one, 2 and 3 weeks after operation. Those results were as follows:

There, though only in the cases which were manipulated thalamotomy alone, occurred an uniform change; a drop of blood pressure. This finding may be explained by Fig. 1 which showed for convenience sake the data from 19 cases; in Group T, a drop of the maximum values from 20 to 40 mm is recognized, and also, even in the minimum values, a drop from 10 to 30 mm, excepting of several cases. For example, in Case 3

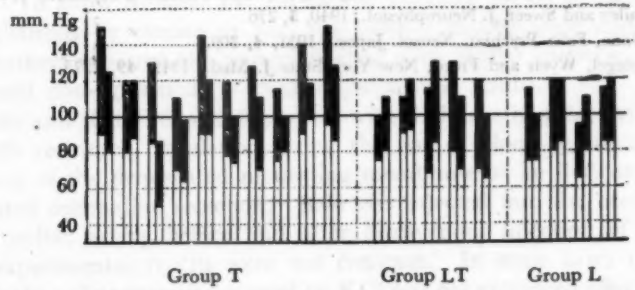

Fig. 1. Results of measurement of blood pressure in the first one week after operation. Subjects consist of 10 cases which were manipulated thalamotomy (Group T); 5 cases, thalamotized after performance of prefrontal lobotomy made 3, or more, months ago (Group LT) and of 4 cases, lobotomized alone (Group L). Squares, in which the upper level is the maximum value and the under, the minimum one, with oblique lines indicate the values recorded before operation and black squares, those after operation.

where a drop from average values of 150/85 to 87/50 in mm Hg occurred. However, such a tendency to decline was transient, since the results from measurements performed after the first one week showed a gradual decreasing. In the first 4 weeks, almost all cases became to show the values fairly equal with those recorded before operation. This item might be accord with the results from stereoencephalotomized thalamotomy which has been reported by Spiegel, Wycis et al.[4]

Differed from the above described findings, the results from the cases which were thalamotized in adding to prefrontal lobotomy, or only lobotomized, were various, since there were remarkable differences case to case (s. Fig. 1). Of course, even in those cases, a tendency to come back within 3 or 4 weeks to the initial level in average values was recognized.

Although the change of blood pressure recovers thus from influenced condition within almost one month, at longest, after operation, its mechanism seems obscure. As one of gensis of such a drop of average values, an influence due to somatic consumed condition, or inertia, which occurs transiently in almost all cases, is also considerable, however its definite conclusion must be looked for furthermore experimental investigations.

Conclusion.

A transient, but relatively marked, drop of blood pressure was observed in the first one week after operation, and at the end of one month after operation, this change was used to come back to the initial level recorded before operation according with its gradual recovery.

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The Effect of Eserine upon the Accelerated Adrenaline Secretion Caused by KCl.*

By

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Having an intimate relation with acetylcholine, potassium ion is considered as an important factor in the synaptic transmission of nerve impulses. It was demonstrated that the potassium ion causes the liberation of acetylcholine when applied to the sympathetic cervical ganglion,[1] submaxillar gland, tongue, sweat gland[2] and heart.[3]

Feldberg and Vartiainen[4] observed that the action of KCl on the perfused cervical sympathetic ganglion was enhanced to some degree after perfusion with eserine. According to Feldberg and Guimarais,[2] the secretory effect of KCl on the submaxillar gland increases definitely after injection of eserine.

KCl has an accelerating action upon the adrenaline secretion, which was demonstrated on non-anesthetized dogs in our laboratory by Arai *et al.*[5] To know whether the adrenaline secreting action of KCl is likewise enhanced by eserine or not, the present studies were undertaken. Some authers reported the papers dealing with similar question, but we had found non-agreement in their experimental results.

Katz and Katz[6] experimented on with dial anesthetized, eviscerated cats with restricted circulation, using the rise of blood pressure and a retraction of the denervated nictitating membrane as an indicator of an augmented adrenaline secretion. KCl was injected into the central end of the coeliac artery before and after intravenous injection of eserine. Their experimental results were not constant. In some cases the augmented adrenaline secretion caused by KCl was not enhanced after eserine. However in some experiments eserine enhanced the action of KCl definitely.

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* Reported at the XX Annual Meeting of the Japanese Physiological Society at Taihoku, July 1941. (Nihon Seiri Z., 1941, **6**, 141; Jap. J. Med. Sci., Biophysics, 1943, **8**, 16.*)

In the same year, Kohler and Lévy[7] reported the accelerating action of KCl upon the adrenaline secretion seemed to be not enhanced with eserine.

On the other hand, Hermann *et al.*[8] noticed a remarkable increase of the adreno-secretory action of KCl after eserine injection. Using the method of the suprareno-jugular anastmosis, KCl was injected intravenously into the donor dog with the acutely or chronically denervated suprarenals before or after eserine injection. The elevation of blood pressure of a recipient dog caused by KCl after eserine was definitly intensiver than that before eserine.

In our experiments the effect of eserine upon the adrenaline secretory action of KCl was studied quantitatively on non-anesthetized dogs.

Method.

In dogs the D_{11}-L_3 dorsal spinal roots were cut under morphine-ether-narcosis, and about 4–11 weeks later the experiments were carried out. The suprarenal vein blood was sampled by using the lumbar route method of Satake *et al.*[9] without anesthesia, and the adrenaline in the suprarenal vein blood was estimated by means of the rabbit intestine segment method. The blood pressure was registered by a mercury manometer from the femoral artery. A small cannula for injection was inserted in the saphena vein.

KCl was injected in a dose of 30–38 mg per kg of body weight as a 3–10% solution intravenously in about 15 seconds. Just after the start of KCl-injection the sampling of suprarenal vein blood was started. The same dose of KCl was injected again 0.5–1 hour after the first KCl injection and some minutes before the second KCl-injection eserine was injection. Eserine salicyl. (Merk) was as a 0.05% solution in a dose of 0.3 mg per kg in 60 seconds intravenously injected. As a control experiment, eserine was not used in Exp. 1. Only KCl in the same dose was injected intravenously twice with the interval of half an hour.

Results.

To show the outline of the experimental procedures, an example (Exp. 5) is given in full in Tab. I. with the protocol the blood pressure tracings are produced in Fig. 1.

Exp. 1 (*Control experiment*) A male dog, 18.5 kg (9. V. 1941)

In this dog, KCl was injected intravenously in a dose of 33 mg per kg in 15 seconds. The animal excited slightly. The blood pressure fell transitorily from 87 mm Hg to 74 mm Hg within 13 seconds after the start of injection

but soon it returned to the initial height. Some seconds later, the blood pressure began to fall promptly and 6 seconds later its lowest height of 47 mm Hg was reached. The rate of heart beat decreased. Then the blood pressure rose and resumed its initial height within 43 seconds after the start of injection.

The adrenaline secretion rate, which was 0.02–0.03γ per kg per minute from one gland before injection, increased after KCl injection to 0.35γ per kg per minute in the first thirty-second-period. In the second thirty-second-period, the secretion rate was somewhat larger than the initial rate but in the third thirty-second-period it was almost the initial value.

About half an hour, 33 mg KCl per kg of body weight was again injected intravenously in 15 seconds. The dog excited slightly. The blood pressure fell temporarily (during about 4 seconds) within 12 seconds after the start of injection. Some seconds later, the blood pressure began to fall from 94 mm Hg, and 4 seconds later it reached 53 mm Hg. The slowing of the heart beat occurred. Then the blood pressure turned to rise and recovered its initial height 31 seconds after the start of injection.

The adrenaline secretion rate before the second KCl injection was 0.02γ per kg per minute. On the injection, it increased to 0.38γ per kg per minute in the first thirty-second-period. In the second thirty-second-period, it resumed almost the pre-injection rate.

In this control experiment we ascertained that KCl in the same dose evoked the acceleration of adrenaline secretion of almost the same intensity.

Exp. 2 A male dog, 11.0 kg (19. IX. 1940)

KCl was injected intravenously in a dose of 30 mg per kg in 17 seconds. Excitement was not observed, but the pupils dilated. Within 9 seconds after the start of injection, the blood pressure fell temporarily from 95 mm Hg to 72 mm Hg and soon it returned to the initial height. The adrenaline secretion rate, which was 0.03γ per kg per minute before the KCl injection increased to 0.39γ. But soon the adrenaline secretion rate resumed almost the pre-injection value.

About an hour after the first KCl injection, 0.3 mg per kg of eserine was injected intravenously in 60 seconds. The pupils dilated. After the end of injection, the blood pressure rose gradually. The adrenaline secretion rate was 0.03–0.04γ per kg per minute, within 4 minutes after eserine injection. 6 minutes later, the animal salivated and urinated.

11 minutes after eserine, 30 mg KCl per kg was again injected intravenously in 18 seconds. The blood pressure did not fall, but rose gradually after the end of injection. The adrenaline secretion rate was measured as 1.1γ per kg per minute in the first thirty-second-period. With the next specimen, the secretion rate was measured as a double of the initial value, but thereafter it resumed nearly the rate of pre-injection.

Exp. 3 A female dog, 13.3 kg (8. X. 1940)

KCl was injected intravenously in a dose of 33 mg per kg of body weight in 15 seconds. The dog did not excite. Within 17 seconds after the start of injection, the blood pressure rose from 87 mm Hg to 98 mm Hg, but soon it began to fall and 9 seconds later its lowest height (55 mm Hg) was reached. The slowing of the heart beat occurred. Then the blood pressure rose up to 77 mm Hg within 36 seconds after the start of injection. The initial rate of adrenaline secretion of this dog was 0.02–0.03 γ per kg per min. On the injection, the secretion rate increased to 0.22 γ per kg per minute during the first thirty-second-period. With the specimen, which was collected in the second thirty-second-period, the rate of adrenaline secretion was about double as much as that of initial value but in the third thirty-second-period it resumed almost the pre-injection value.

About half an hour later, eserine was injected intravenously in a dose of 0.3 mg per kg of body weight in 60 seconds. Soon after eserine pupils dilated and the respiratory movements became deep and rough. Shivering was observed. The blood pressure rose gradually. The rate of the heart beat rather decreased. 5 minutes after eserine injection, the adrenaline secretion rate was 0.03 γ per kg per min.

8 minutes after eserine injection, 33 mg per kg KCl was again injected intravenously in 16 seconds. The animal urinated and defectaed. The blood pressure rose gradually from 118 mm Hg and reached 142 mm Hg within 23 seconds after the start of injection. The adrenaline secretion rate increased definitely after the injection. In the first thirty-second-period, it was 1.7 γ per kg per min., and in the next thirty-second-period the secretion rate was 0.17 γ per kg per minute. With the specimen taken in the third thirty-second-period, it was still over the pre-injection value.

Exp. 4 A male dog, 19.2 kg (26. XI. 1940)

In this dog, KCl was injected intravenously in a dose of 38 mg per kg in 17 seconds. Within 13 seconds after the start of injection the blood pressure rose from 88 mm Hg to 98 mm Hg, but soon it began to fall and reached 56 mm Hg within 25 seconds after the start of injection. The rate of heart beat decreased. Then the blood pressure turned to rise and elevated to 92 mm Hg. The basal secretion rate of adrenaline was 0.03 γ per kg per min. On the KCl injection, the adrenaline secretion rate was found to have increased in the first thirty-second-period to 0.26 γ per kg per min. In the second thirty-second-period it was still somewhat larger than the initial value.

About half an hour after the first KCl injection, 0.3 mg per kg of eserine was injected in 60 seconds. The pupils dilated. The blood pressure rose from 88 mm Hg gradually after the end of injection. 2 minutes later 175 mm Hg was reached. The secretion rate of adrenaline was 0.04 γ per kg per minute. Then 38 mg per kg of KCl was again injected intravenously in 17 seconds. The blood pressure began to fall from 180 mm Hg within 20 seconds after the start of injection and 5 seconds later it reached 124 mm Hg. The rate of heart beat decreased. Then the pressure began to rise and reached

176 mm Hg. The adrenaline secretion rate in the first thirty-second-period was 0.7 γ per kg per min. In the second thirty-second-period the secretion rate resumed nearly its pre-injection value.

Exp. 5 A female dog, 15.4 kg (3. XII. 1940)

The basal adrenaline secretion rate in this dog was 0.02 γ per kg per minute. KCl was injected intravenously in a dose of 35 mg per kg in 15

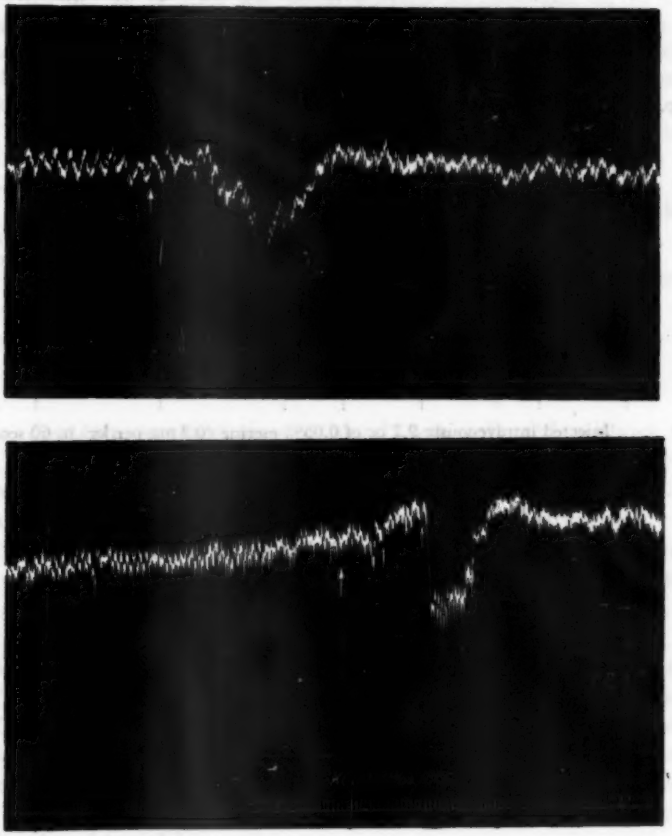

Fig. 1. Blood pressure tracings in Exp. 5. (Reduced to 3/5) Dog 15.4 kg ♀, non-anesthetized.
KCl was injected intravenously in a dose of 35 mg per kg of body weight.
(I) KCl injection before eserine.
(II) KCl injection after eserine.
Time in seconds.

TABLE I.

Exp. 5. 3. XII. 1940 Dog ♀ 15.4 kg

Time	Time after KCl injection (sec.)	No. of specimen	Blood flow per kg per min. (cc)	Adrenaline in 1 cc blood (γ)	Adrenaline output per kg per min. (γ)	Blood pressure (mm Hg)	Heart rate (per min.)	Respiration (per min.)	Body temperature (C°)
31. X. 1940 15.3 kg D_{11}–L_3 dorsal spinal roots severed. 9:50 a. m.–11:40 a. m. The left lumbar route preparation. The left femoral artery and the right saphena vein prepared:									
1:36 p.m.									39.0
1:45		1	0.49	0.05	0.02	86	150	30	
		2	0.41	0.05	0.02				
1:48									38.5
1:50		3	0.35	0.05	0.02	82	135	24	
1:55									38.3
2:10		4	0.43	0.05	0.02	85	159	23	
2:11	Injected intravenously 9.2 cc of 0.05% KCl (35 mg per kg) in 15 seconds.								
	0–30	5	0.57	1.0	0.57	85–39–66	168–60–168		
	30–60	6	0.53	0.05	0.03	66–35	168	30	
	60–90	7	0.56	0.05	0.03	85	168	26	
2:22									38.8
2:32		8	0.41	0.05	0.02	71	162	30	
		9	0.39						
2:33	Injected intravenously 9.2 cc of 0.05% eserine (0.3 mg per kg) in 60 seconds.								
2:33						68	150	27	
2:36						98	132	30	
2:37		10	0.60	0.05	0.03	112	132	51	
	Injected intravenously 18 cc of 3% KCl (35 mg per kg) in 16 seconds.								
	0–30	11	0.95	2.0	1.9	126–74–94	168–96–110		
	30–60	12	0.79	0.2	0.16	94–128–121	110–171	11	
	60–90	13	0.60	0.05	0.03	125	153	11	
2:45						112	168	30	39.0
2:47		14	0.51	0.05	0.03	111	171	52	
		15	0.49						
2:55						104	177	48	39.1
3:04						88	153	42	
3:09		16	0.41	0.05	0.02	92	162	45	
		17	0.43						
3:11									39.2
Room temperature 17–18 °C.									

seconds. During the injection the animal barked. The blood pressure began to fall from 85 mm Hg within 12 seconds after the start of injection and 11 seconds later its lowest level (39 mm Hg) was reached. The rate of the heart beat decreased markedly. Then the blood pressure rose and 13 seconds later it returned almost to the initial height. The rate of adrenaline secretion measured

with the specimen taken in the first thirty-second-period was 0.57 γ per kg per minute. In the second thirty-second-period, the rate of secretion resumed the initial value.

About twenty minutes after the KCl injection, eserine was injected intravenously in a dose of 0.3 mg per kg in 60 seconds. After finishing the eserine injection, the pupils dilated and the respiration became rough and deep. The blood pressure rose from 68 mm Hg gradually. Two minutes later, the animal struggled. The adrenaline secretion rate was 0.03 γ per kg per min.

35 mg per kg of KCl was again injected intravenously in 16 seconds. The animal shivered. The blood pressure fell promptly from 126 mm Hg to 74 mm Hg within 20 seconds after the start of injection and lasted for 8 seconds. Slowing of the heart beat occurred. Then the blood pressure rose and returned to initial height 33 seconds after the start of injection.

The adrenaline secretion rate accelerated markedly after injection. It was 1.9 γ per kg per minute in the first thirty-second-period. In the second thirty-second-period, the rate of adrenaline secretion was 0.16 γ per kg per min. In the third thirty-second-period the secretion rate of adrenaline resumed the pre-injection value.

From the description quoted above, it is known that the accelerating action of KCl upon the adrenaline secretion of the suprarenals is enhanced by eserine.

Summary.

In dogs, the suprarenal vein blood was collected without narcotizing. Adrenaline in the blood specimen was determined by means of the rabbit intestine segment method.

At first, 30–38 mg per kg of KCl was injected intravenously in 15 seconds. After about half an hour, 0.3 mg per kg eserine was injected intravenously in 60 seconds, and 4–11 minutes later KCl in a same dose was again injected. The adrenaline secretion after the second KCl injection was compared with that of the first KCl injection.

Before injection, the rate of adrenaline secretion from one gland was 0.02–0.03 γ per kg per min. At the first KCl injection, it increased to 0.22–0.57 γ per kg per min. After eserine, and before the second KCl injection, the rate of adrenaline secretion was 0.03–0.04 γ per kg per min. At the second KCl injection, it increased to 0.7–1.9 γ per kg per min.

Thus it is clear that the accelerating action of KCl upon the adrenaline secretion is enhanced by eserine.

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How Acts the Evipan-Sodium Anesthesia upon the Adrenaline Secretion of the Suprarenal Glands?*

By

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(Received for publication, March 27, 1951)

In the present investigation we attempted to know the effect of evipan-sodium anesthesia upon the adrenaline secretion. There are only few references concerning this question.

Using the method of suprareno-jugular anastomosis in dogs Tournade and Joltrain[1] injected evipan-sodium intravenously to the donor dog, and observed a fall of the blood pressure and an enlargement of the spleen volume of the recipient dog. Thereby they came to the belief that the evipan-sodium anesthesia inhibits the spontaneous adrenaline secretion from the suprarenal gland. They further observed the diminution of the accelerating effect of stimulating the peripheral cut end of the splanchnic nerves upon the adrenaline secretion.

The effect of some anesthetics upon the adrenaline secretion of the suprarenal gland is considerable. The acceleration of adrenaline secretion in morphine[2] anesthesia and urethane[3] anesthesia is well established. On the other hand it has been proved that non-acceleration of adrenaline secretion occurs in chloralose,[4] avertin[5] and veronal[6] anesthesia.

Methods.

Dogs were used. In order to collect the suprarenal vein blood without anesthesia, fastening and evoking any pain, the D_{11}-L_3 dorsal spinal roots were sectioned under morphine-ether anesthesia. Four weeks or more after the spinal roots operation, the present experiments were carried out. The lumbar route operation of Satake *et al.*[7] was performed and the suprarenal vein blood specimen was collected at first without anesthesia. After some specimens

* Shortly reported at the XXII Annual Meeting of the Japanese Physiological Society at Fukuoka, March 1943. Nihon Seiri. Z., 1943, **8**, 551; Jap. J. of Med. Sci., Biophysics, 1944, **9**, 77.

were collected as a control in non-anesthetized state, evipan-sodium was injected. And thereafter the blood specimen was collected from time to time. The adrenaline in the suprarenal vein blood specimen was estimated by means of the rabbit intestine segment method.[8]

The blood pressure was registered from the femoral artery by the Hg-manometer. The blood sugar content was estimated by means of Hagedorn and Jensen.

Evipan-sodium (Bayer) was injected as a 10% watery solution in a dose of 40–50 mg per kg intravenously in 2 minutes.

Results.

The results from 4 dogs were condenced in Table I and the outline of the experimental procedure was showed in Table II.

Evipan-sodium in a dose of 40 mg per kg was injected into Dog 1. During injection the animal became sleepy and laid down by the side. The nictitating membrane protruded largely. This state lasted over one hour. The blood pressure fell gradually during injection from 107 mm Hg to 80 mm Hg. Even about 2 hours later it did not resume the initial height, although the tendency of a very gradual recovering was observed. The rate of heart beat increased after evipan-sodium injection.

The rate of adrenaline secretion before evipan injection was measured as 0.01 γ per kg per minute from one gland. 10 minutes after injection the blood flow through the suprarenal gland became somewhat smaller and the rate of adrenaline secretion was estimated as 0.005 γ per kg per minute. The blood samples collected about 50, 60, 75 and 90 minutes after injection did not show any definite alteration of adrenaline secretion rate in comparing with the initial rate. It was 0.009–0.02 γ per kg per minute. The increase of adrenaline secretion in an insignificant degree was observed 2 hours after evipan injection, i. e. 0.03 γ per kg per minute.

No definite effect of evipan sodium anesthesia upon the blood sugar content was also observed. It was 0.084–0.087% before injection and 0.074–0.080% after evipan. The body temperature fell from 37°.0 C. to 35.°2 C. and about one hour after injection it tended to recover very slowly.

Similar results were obtained in Dog 2. The protocol is given as example (Table II). In this case 50 mg per kg evipan-sodium was injected intravenously. The animal was anesthetized deeply before the end of injection. Duration of deep anesthesia was about one hour. The blood pressure fell from 91 mm Hg to 46 mm Hg and then it elevated step by step. The heart rate increased slightly. The pre-injection rate of adrenaline secretion was 0.03 γ per kg per minute. After injection it was 0.009–0.03γ. No definite variation was observed in the blood sugar

TABLE I.

TABLE I.

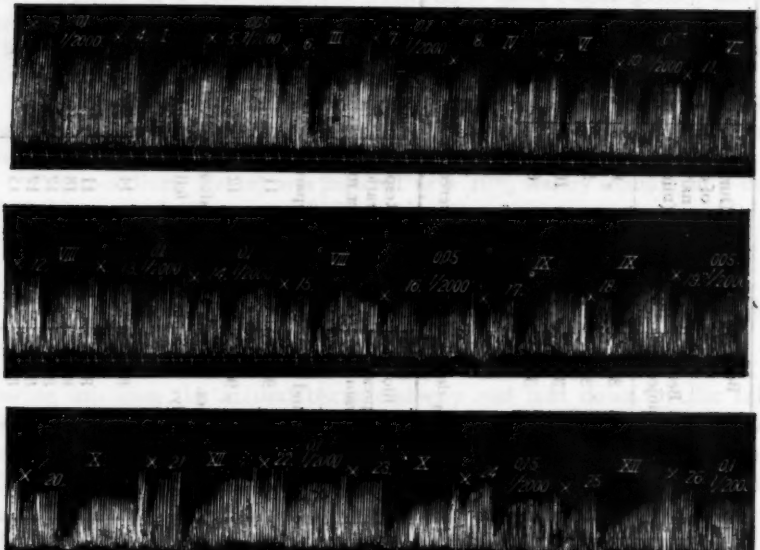
The Adrenaline Output, Blood Pressure, Blood Sugar, etc. in Dogs, after Intravenous Injection of Evipan-sodium.

No. of dog	Date (1942)	Body weight and sex	Dose of evipan-sodium per kg (mg)	Adrenaline output per kg per min. (γ)		Blood pressure (mm Hg)		Blood sugar (%)		Body temperature (°C.)		Duration of deep narcosis (minute)	Room temperature (°C.)
				Before injec.	After injection	Before injection	After injection	Before injection	After injection	Before injection	Minimum after injection		
1	9.X.	12.7 ♀	40	0.01	0.005–0.03	107	80–90	0.084	0.074–0.080	37.0	35.2	80	19–20
2	13.XI.	15.7 ♂	50	0.03	0.009–0.03	91	46–89	0.103	0.091–0.108	39.0	37.5	50	16
3	20.XI.	17.7 ♂	50	0.03	0.02–0.04	89	28–82	0.103	0.095–0.118	39.3	37.1	100	15–16
4	27.XI.	10.3 ♀	40	0.02	0.01–0.02	72	53–76	0.108	0.090–0.102	38.4	37.2	60	12–13

TABLE II.

Dog 2. 13. XI. 1942 15.7 kg ♂ 11. IX. 1942 17.3 kg D_{11}-L_2 dorsal spinal roots severed.

Time	No. of blood specimen	Quantity (cc)	Duration of collection (sec.)	Blood flow per kg per min. (cc)	Adrenaline in 1cc blood (γ)	Adrenaline output per kg per min. (γ)	Blood sugar (%)	Blood pressure (mm Hg)	Heart rate (per min.)	Respiration (per min.)	Body temperature (°C.)
	10:45 a. m.—1:34 p. m. L. lumbar route preparation; L. femoral artery and r. saphena vein prepared.										
3:30 p.m.	I	1.4	30	0.31	0.1	0.03	0.110	95	150	11	39.0
	II	2.8	30	0.36							
3:40	III	2.2	30	0.28	0.1	0.03	0.103	96	156	12	
3:48–3:50	Intravenous injection of 7.9 cc 1% evipan-sodium (50 mg per kg) in 2 minutes. About 40 seconds after the start of injection the respiration became rough. But soon the animal slept profoundly. The blood pressure fell from 91mm Hg gradually and 46 mm Hg was reached at the end of injection.										
3:55	IV	1.4	30	0.18	0.1	0.02	0.108	85	162	14	38.3
	V	1.7	30	0.22							
4:05	VI	1.9	30	0.24	0.1	0.02	0.110	85	162	11	
4:20	VII	1.5	30	0.19	0.1	0.02	0.105	89	168	18	37.8
4:30	VIII	2.4	60	0.15	0.1	0.02	0.105	84	180	12	37.6
4:45	IX	2.9	60	0.18	0.05	0.009	0.108	84	186	12	37.5
5:00	X	1.7	30	0.22	0.15	0.03	0.103	85	192	15	37.7
	XI	2.2	30	0.28							
5:30	XII	1.4	30	0.18	0.1	0.02	0.091	84	192	16	37.7
	Room temperature 16°C. 3:50—4:40 p. m. Deep anesthesia. 4:45 Shivering.										



Intestine tracings for Dog 2. (Reduced to 3/5)

In all tracings, at the mark "×" atropine-Tyrode's solution in which the rabbit intestine segment was beating rhythmically, was replaced by indifferent blood solution, and at the "numeral" by the indifferent blood solution to which a certain quantity of adrenaline chloride of Sankyo Co. was added, or by the specimen solution. All the blood solution were prepared by diluting with 4 volumes of Tyrode's solution, and the quantity of the blood employed for an assay was 0.5 cc. Time intervals are 30 seconds.

"0.1 1/2000" shows "0.1 cc. of adrenaline chloride solution with the concentration of 1/2000 mg in 1 cc.," i. e. 0.05 γ adrenaline chloride.

I: About the same with 0.05 γ and stronger than 0.025 γ.

III: Stronger than 0.025 γ and about the same with 0.05 γ.

IV and VI: About the same with 0.05 γ and stronger than 0.025 γ.

VII and VIII: Almost as strong as 0.05 γ. IX: About the same with 0.025 γ. X: A little stronger than 0.05 γ and almost as strong as 0.075 γ. XII: A little weaker than 0.075 γ and about the same with 0.05 γ.

To sum up:—

Specimen I, spec. III, spec. IV, spec. VI, spec. VII, spec. VIII, and spec. XII were assayed as 0.1 γ in 1 cc.

Spec. IX was assayed as 0.05 γ in 1 cc. and spec. X as 0.15 γ in 1 cc.

content after evipan injection. It was 0.100–0.110%. Only a small decrease of blood sugar was found 1.5 hour after injection. The body temperature fell from 39.°0C. to 37.°5C. after evipan.

Almost the same result was obtained in Dog 3. In this dog, evipan-

sodium in a dose of 50 mg per kg was injected. The blood pressure fell from 89 mm Hg to 28 mm Hg after injection and then it ascended again. The increase of the heart rate was also observed. The rate of adrenaline secretion did not show any noticeable alteration. The initial rate was 0.03 γ per kg per minute and it fluctuated after injection from 0.02 γ to 0.04 γ per kg per minute. The blood sugar content remained nearly unaffected by evipan-sodium injection. The body temperature descended also in this dog from 39°.3C. to 37°.1C.

On Dog 4 evipan-sodium was injected in a dose of 40 mg per kg. The animal was deeply anesthetized. The blood pressure fell slightly after evipan-sodium. The rate of heart beat increased. The initial rate of adrenaline secretion was in this case 0.02 γ per kg per minute. With the samples collected just before and just after the end of injection the rate of adrenaline secretion was measured as 0.02 γ and 0.01 γ per kg per minute. Thereafter it remained almost unchanged. A small decrease of blood sugar content such as from 0.108% to 0.090% was observed in this case. The body temperature descended from 38°.4C. to 37°.2C.

Thus we could not find any appreciable alteration of adrenaline secretion after evipan-sodium injection. From our experimental results it is clear that evipan-sodium does not stimulate the adrenaline secretion from the suprarenal glands. But it is not easy to determine in our experiments, whether the evipan-sodium anesthesia diminishes the resting secretion of adrenaline or not, as the adrenaline content of suprarenal vein blood in the resting state or during the evipan-sodium anesthesia is very small and is almost the minimum-limit of measurable quantity of adrenaline by the rabbit intestine segment method.

Non-influence of evipan-sodium anesthesia upon the blood sugar content had been reported by some previous experimentalists, such as Weese,[9] Kennedy & Narayana[10], and Atnan & Fenz.[11] The results of our present experiment coincides well with those reports on this point. Quite the same results were obtainable in 5 other dogs, as given in Tab. III. Only Prasad & Sen[12] observed the hyperglycaemia after evipan-sodium injection.

Before we performed the above described experiments, evipan-sodium was injected in 5 normal dogs to know the dose of evipan-sodium for use. The blood pressure was registered from the left femoral artery by Hg-manometer. The blood sugar content was estimated by Hagedorn & Jensen. Evipan-sodium was injected as a 10% watery solution intravenously in a dose of 20 mg, 30 mg, 40 mg, 50 mg and 80 mg per kg. The results were presented in Table III.

In Dog 9, which received 20 mg evipan-sodium per kg, the anesthesia lasted only 4 minutes. Convulsion-like movements took place. The evipan-

TABLE III.

The Blood Sugar and Blood Pressure etc. in Dogs, after Intravenous Injection of Evipan-sodium.

No. of dog	Date (1942)	Body weight (kg) and Sex	Dose of evipan-sodium per kg (mg)	Blood sugar (%)		Blood pressure (mm Hg)		Body temperature (°C.)		Duration of deep narcosis (min.)
				Before injection	After injection	Before injection	After injection	Before injection	Minimum after injection	
5	9. IX.	10.2 ♂	50	0.107	0.093–0.102	73	36–88	38.5	37.9	65
6	16. IX.	7.2 ♂	30			87	57–92	39.0	37.9	40
7	23. IX.	7.8 ♀	40	0.099	0.078–0.102	93	54–110	39.4	37.3	45
8	24. X.	5.2 ♂	80	5 minutes after injection the animal died.						
9	24. X.	11.0 ♂	20	0.096		98		38.1		4
			20*		0.084–0.114		85–116		36.1	20

* 5 minutes after the first injection 20 mg per kg evipan-sodium was further injected.

sodium was further injected in a dose of 20 mg per kg intravenously. Deep anesthesia developed, which lasted about 20 minutes.

In Dog 8, evipan-sodium was injected in a dose of 80 mg per kg. Soon after the start of injection the blood pressure dropped promptly, and some minutes after the injection the animal died eventually. In other 3 dogs, evipan-sodium was applicated in a dose of 30 mg, 40 mg and 50 mg per kg respectively. The deep anesthesia lasted from half to one hour. The fluctuations of blood sugar content and blood pressure was similar as was observed in Dog 1–4. The body temperature descended also slightly.

Weese[9] studied on the effective dose of evipan-sodium in some species of animals. According to him, the minimum-hypnotic dose for dogs is 25 mg per kg, the minimum-narcotic dose is 30 mg per kg and the minimum-lethal dose is 100 mg per kg. Imaoka *et al.*[12] reported that the suitable dose of evipan-sodium for dogs is 30–40 mg per kg and the minimum-lethal dose is 80 mg per kg. Therefore our results are in complete accord with those of Weese and Imaoka *et al.*

SUMMARY.

The suprarenal vein blood of dogs was collected by the method of Satake *et al.* without fastening and laparotomy and at first without anesthesia. Then evipan-sodium was injected intravenously in a dose of 40–50 mg per kg. The adrenaline in the suprarenal vein blood was estimated by the rabbit intestine segment method. The blood sugar content was also estimated.

The evipan-sodium anesthesia does not cause any significant alteration of adrenaline secretion rate and of blood sugar content.

The effective and minimum-lethal dose for dogs are given in the text.

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Studies on the Antibiotic Substances from Actinomyces.

(13th report)

By

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(Received for publication, May 29, 1951)

Ishida of our laboratory has isolated a new basic streptothricin-like, but non-toxic substance " Reseomycin " from a strain of *Streptomyces roseochromogenus*. This substance shows a higher activity against *Vibrio comma* than streptomycin in vitro, as well as in vivo. The authro made a comparative study, dealing with the activities of roseomycin, steptomycin, chloromycetin, sulfathiazole and malfanil against *Vibrio comma*, in vitro, and roseomycin, chloromycetin and malfanil in mice inoculated with this organism and obtained following results:

1) Against 10 strains of *Vibrio comma*, these substances were effective in vitro in the following order: chloromycetin, roseomycin, sulfathiazole, malfanil, and streptomycin.

2) In experimental infections, roseomycin and chloromycetin showed a similar effect.

The Tohoku Journal of Experimental Medicine, Vol. 54, No. 3, 1951.

Study of the Technique of the Radiographic Delineation of the Cross Section of the Body.

(Study on Rotatography and Crossgraphy)

By

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(Received for publication, March 28, 1951)

E. Lysholm[1] (1926) began the development of Palmieri's "Radioplastic" to study the body's appearance in cross section using a drawing device to trace the fluoroscopic image on paper. After some time the method fell into disuse.

In 1942 Prof. Yoshihiko Koga* began using another method to study the chest in which multiple exposures were made with the body rotated systematically through numerous angles. At the time the name rotating radiography was used. Using this idea I have developed four methods and the equipment for the radiographic study of a cross section of the body at any level. These methods have been called Discontinuous Rotatography,[2] Continuous Rotatography,[2] Direct Crossgraphy[3] and Indirect Crossgraphy. These methods differ and will be described individually.

Rotatograph—Construction and Operation.

Common to all four methods is the device, Rotatograph, with which the roentgen exposures are made. Two types of Rotatographs are used at present.

The first, *Horizontal Rotatograph*, is illustrated and described in Fig. 1. To make a cross section examination the level of the part to be studied is identified by flouroscopy or conventional roentgenograms and marked with ink. The patient is immobilized and the machine is adjusted so that the center of rotation of the part at the point to be sectioned and the cross wire on the slit in the lead diaphragm over the film are all lined up exactly in the central ray. The height of the tube arm is adjusted so that its center of rotation is in the same plane as the center of the part at the point to be sectioned.

* Director of the Department of Radiology, Tohoku University School of Medicine, Sendai.

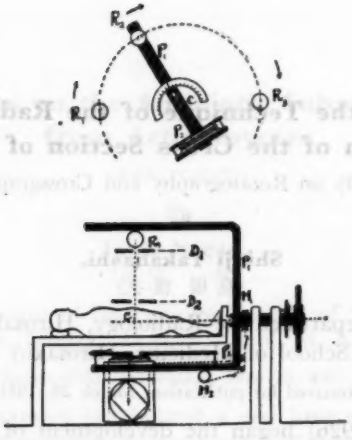

Fig. 1. A schematic drawing of the horizontal rotatograph. At one end of examining table is a fulcrum bearing a rotating U shaped arm. One end of the arm holds the x-ray tube R and the other end supports the cassette tunnel benath the table. Degrees of rotation are read at the rotation center. The axis of the rotation center and the central x-ray beam narrowed to a 1 or 2 cm band by slits in diaphragm D_1 and D_2 pass through the center C of the patient at the level studied. The top of the cassette tunnel is lead covered and contains a transverse slit 22 inches long and 10 mm wide for discontinuous and 2 mm wide for continuous rotatography. Across the center of this slit is an opaque wire. The cassette is held on a holder which slides in the cassette tunnel pulled automatically by cord over pulleys. M_1 and M_2. The holder also rotates on an imaginary axis formed by the central ray.

In Discontinuous Rotatography multiple exposures are made at different degrees of rotation. To begin an examination the tube and cassette arms are rotated to a horizontal or 0 degrees position and an exposure is made. The tube and cassette arms are rotated, usually 10 degrees, and the film slides in the cassette holder tunnel sufficiently, 10 mm, to bring a new piece of film beneath the diaphragm slit and a second exposure is made. The arms again are rotated, the film is moved to a new area and a third exposure is made. This is repeated through an arc of 180 degrees. The film is then developed.

In Continuous Rotatography the arms move without stopping thru the same arc of 180 degrees with constant roentgen exposure during the rotation. At the same time the film moves steadily beneath the 2 mm wide lead diaphragm slot at the rate of one millimeter for every one degree of tube rotation. If an extremity or the chest is examined the other extremity and the arms must not be in the beam during rotation and are usually elevated out of the way before starting.

The second is the *Vertical Rotatograph* and is illustrated and described

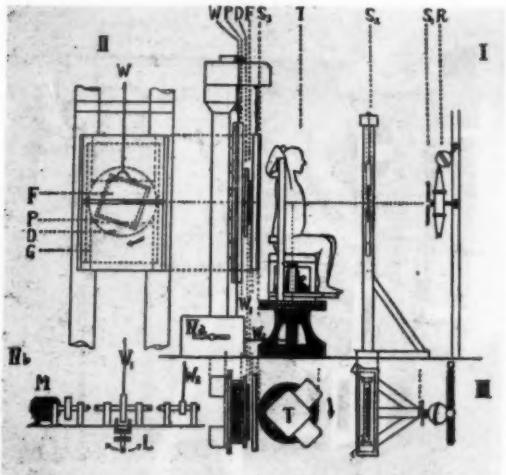

Fig. 2. A schematic drawing of the vertical rotatograph (see Fig. 3). Tube R is adjustable for height and has a cone with a narrow slit. Another narrow slit in shield S_2 narrows the beam to a 1 or 2 cm band on the patient. The patient sits on a rotating stand T which is adjustable for height. Behind the patient is the cassette tunnel covered by a lead shield S_3 having a 22 inch long lead slit which is 10 mm wide for discontinuous rotatography and 2 mm wide for continuous rotatography. The film cassette F is held in a rotating and sliding mount D in the cassette tunnel. Motor M and wires W_1 and W_2 move this sliding mount up or down and rotate the patient simultaneously. The film also can be rotated manually around an axis formed by the central ray (see arrow.)

in Figs. 2 and 3. The general principle is the same as in the horizontal Rotatograph except that the patient rotates instead of the tube and cassette. An arrangement is made for the cassette to turn on an imaginary center axis perpendicular to the film plane with the center of rotation in the central ray. In making a cross section study the level to be examined is located by fluoroscopy or conventional roentgenograms and marked in ink. The patient is seated on the rotating seat with his right side toward the tube and immobilized with a binder to a back on the seat. If the chest is examined the arms are raised to shoulder height. The level of the seat is adjusted so that the inkmark at the level to be sectioned is directly opposite the slit in the cassette tunnel diaphragm. The tube focus and the slit in the intervening shield are also adjusted to this level. The patient is adjusted on the seat so that the center of the body cross section at the level studied is in the vertical axis of the seat rotation and also in line with the central ray and center wire across the cassette tunnel lead diaphragm slit. From this starting position the patient is rotated thru the A.P. projection in the different types of examinations. The tube

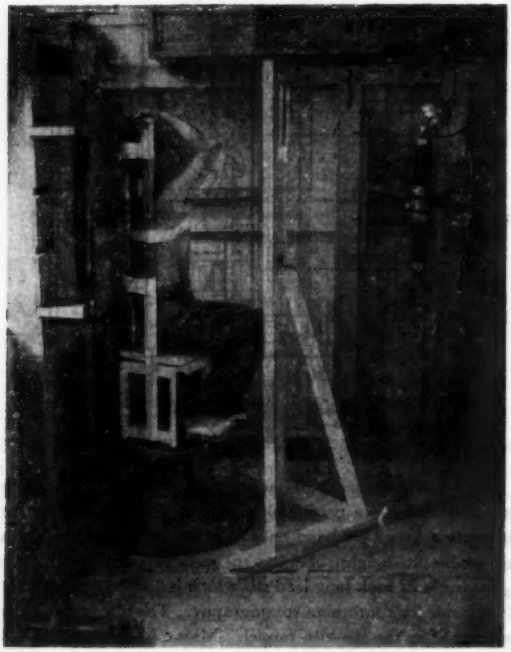

Fig. 3. Vertical rotatograph described in Fig. 2.

does not move during any examination with the vertical rotatograph.

In Discontinuous Rotatography multiple exposures are made at different degrees of rotation. Beginning as described an exposure is made. The patient is rotated usually 10 degrees and the film moved vertically 10 mm across the 10 mm wide slit in the cassette tunnel and a second exposure is made. The patient is again rotated 10 degrees and the film moved 10 mm and a third exposure made. This is continued until the patent is intermittently or discontinuously rotated thru an angle of 180 degrees. The film is then developed. (Fig. 4)

In Continuous Rotatography exposure and rotation occur simultaneously and without interruption or continuously. With the patient positioned as described a motor drive slowly rotates the patient and at the same time slowly moves the film vertically across the 2 mm wide slit in the cassette tunnel diaphragm at the rate of 1 mm for each degree of patient rotation. When the rotation has gone thru an angle of 180 degrees the exposure and motion of the patient and cassette stops. Roentgen exposure is constant from the beginning to the end of the 180 degree rotation. The film is then developed. (Figs. 6–9).

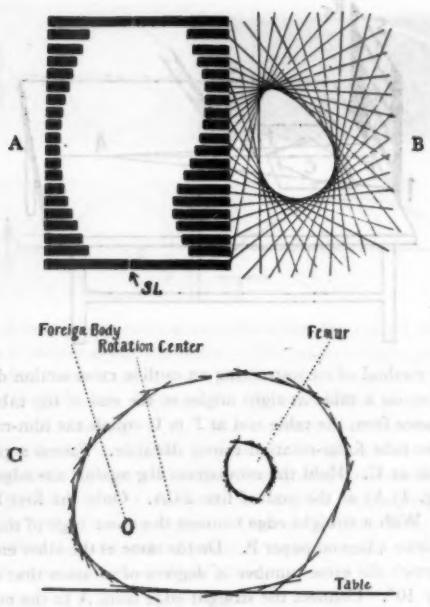

Fig. 4. A. A discontinuous rotatogram made of a piece of lead having sides formed by three curves of different radii and a straight line. Each hand represents an exposure at intervals of 10 degrees rotation of the lead, the total rotation arc being 180 degrees. The light portion in the center is produced by the shadow of the lead. At SL the white line is the standard line formed by the wire across the cassette tunnel diaphragm in the central ray and represents the center of rotation.

B. A reconstructed outline drawing of the cross section outline of the piece of lead used in A. The method of reconstruction is described in Fig. 5.

C. Clinical application of discontinuous rotatography showing the location of an opaque foreign body in the left thigh. The outline of the soft tissue, bone and foreign body was drawn the same as in B. The original drawing was the same size as the thigh.

In Direct Crossgraphy the patient is positioned as described with the right side toward the tube. (Fig. 2, 3) The film is placed in the holder with the long axis of the film parallel to that of the body and the holder raised so that the lower edge of the film is at the slit in the cassette tunnel diaphragm. With the patient immobilized and, if a chest also holding his breath during each exposure the cassette moves down by a motor drive closing a contact which starts the roentgen exposure. After the entire film has steadily moved down behind the 2 mm wide slit in the cassette tunnel diaphragm a contact is broken and the roentgen exposure stops. The patient is then turned on the rotating seat 10 degrees toward the A.P. and the cassette is turned clockwise in the cassette holder

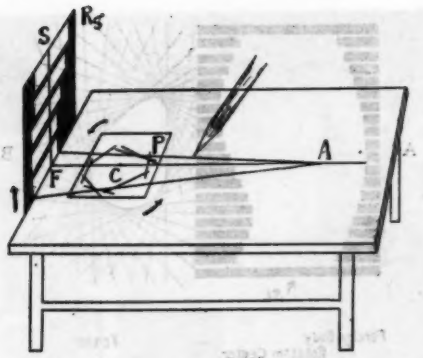

Fig. 5. The method of reconstructing an outline cross section drawing from a rotatogram. Draw a line on a table at right angles to the end of the table and mark points C and A. The distance from the table end at F to C equals the film-rotation center distance and AC equals the tube focus-rotation center distance. Fasten a piece of drawing paper P by a thumb tack at C. Hold the rotatogram Rg against the edge of the table with the standard line (Fig. 4) A) at the end of line FCA. Only the first band exposed projects above the table. With a straight edge connect the outer edge of the shadow of the object with point A and draw a line on paper P. Do the same at the other end of the band. Move the paper (see arrow) the same number of degrees of rotation that each band on the film represents, usually 10°. Connect the straight edge from A to the outer ends of the object shadow in the second band and draw lines on paper P. Repeat this for each band turning the paper each time until an acrc of 180 degrees is reached.

also 10 degrees on an imaginary axis thru the center of the film perpendicular to the film (this axis should coincide with central ray). The film then moves up starting and stopping the exposure before and after it passes behind the slit in the cassette tunnel diaphragm. The patient is again turned 10 degrees and the film also turned 10 degrees. The film then moves down being exposed as before after which the patient and film are again turned 10 degrees. This continues by 10 degrees steps unitl the patient and film have turned thru 180 degrees. The film is now ready for development. Immediately after making the rotatogram an 8×10 film is placed between the patient and cassette tunnel and a standard exposure made in the AP position. A lead marker on the cassette indicates the level of the cassette tunnel diaphragm slit. The film is a permanent record of the level sectioned.

The only motion during exposure is the film moving up or down. This rate of motion is about 3 inches per second but it can be varied depending on the intensity of the roentgen beam and sreen and film sensitivity. Different exposure for the varying thickness as the part rotates is controlled by changing the milliamperes or kilovolts as necessary for each

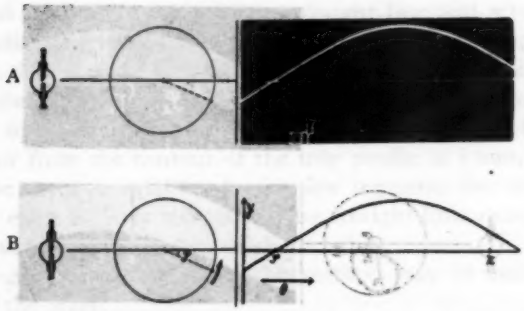

Fig. 6. A. A continuous rotatogram of a point, the curved line is the image of the point projected as it rotates about the center. The white straight line is the standard line and is the image of the wire across the diaphragm slit and represents the center of rotation on the film. The length of this rotatogram represents 180 degrees rotation.

B. Schematic representation of A. The standard line becomes the abscissa and at right angle to this at the left end is the y ordinate. Angle φ is the number of degrees the point rotates to cross the central ray extending through the circle of rotation at the left. The distance from the y ordinate to π represents 180 degrees rotation of the point and one one hundred eightieth part of this is one degree. Thus the distance from y ordinate to φ, angle φ, can be measured in degrees and its position reconstructed. The arrow indicates the direction of object rotation viewed from above.

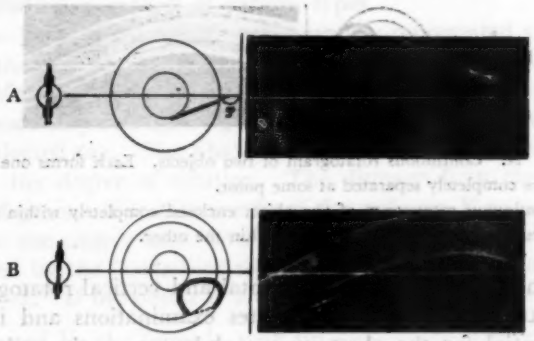

Fig. 7. A. Continuous rotatogram of a straight line. This appears as a curved zone which converges becoming progressively dense at the convergent point. At this point the line object is parallel to the x-ray (not necessarily the central ray). The arc through which the line object rotates to reach this point is angle φ.

B. Continuous rotatogram of a solid with one concave surface. The concavity is bridged by an imaginary line. When this line is parallel to the x-ray a convergent point called a nodal point is formed at T. No progressive increase in density occurs.

exposure. Direct crossgraphy can be done also on the horizontal rotatographe.

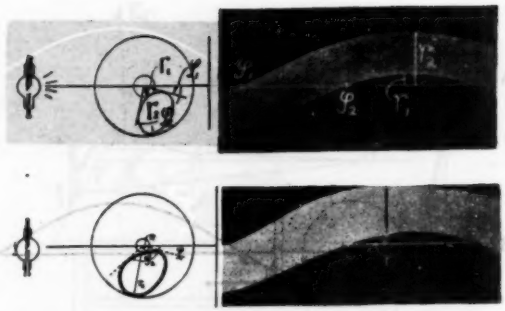

Fig. 8. Continuous rotatogram of a solid. The letters indicate important landmarks or localizing solid objects described in the text and Fig. 6.

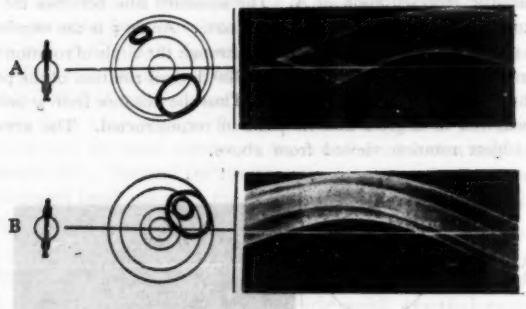

Fig. 9. A. Continuous rotatogram of two objects. Each forms one of tow curved zone that are completely separated at some point.

B. Continuous rotatogram of one object enclosed completely within another. Two curved zones are seen, one is completely within the other.

While the principle of the horizontal and vertical rotatographs is the same the latter more readily facilitates examinations and is preferable but not essential for the chest, upper abdomen, neck, and skull. The horizontal rotatograph can be used for all body parts but we have used it mainly for extremities where it is preferred to the vertical rotatograph.

Appearance and Interpretation of Records.

In Discontinuous Rotatography the rotatogram exposed during rotation of the patient is used to make a drawing in which the silhouettes of the various parts of the object studied are outlined by segments of many straight lines forming the periphery of the drawing. In Figures 4 A is illustrated and described a simple discontinuous rotatogram made of a

piece of lead with sides formed by a straight line and several curves of different radii. From this rotatogram the outline drawing of the cross section (Fig. 4 B) is made according to Figure 5. The profile thus drawn is the same size as the cross section of the original object with the periphery formed, by segments of straight lines. If a segment is 2.4 cm long the greatest error from the contour of the true profile is 1 mm. With many segments the error is small and with few segments the error increases. The geatest error is likely to occur where straight lines exist in the object and the least error with curves of short radii.

By using the same procedure this method may be used for studying any part of the body.

Continuous rotatography.

In the Continuous Rotatogram the "standard line" produced by the lead wire across the cassette tunnel diaphragm slit in the path of the central ray is seen as a white line through the center of the rotatogram where it represents the center of rotation of the part studied (Figs. 6, 7, 8, 9). The shadow of the object studied is seen as a wave-like zone or line. This type of rotatogram is difficult to read directly therefore, to facilitate interpretation, a number of laws were formulated after conducting studies on models of various types.

Law—1. The continuous rotatogram is represented as the rectangular coordinates y and 0 (Fig. 6). Along the ordinate y is measured the distance of the object or point from the standaid line or center of rotation. This distance may be slightly greater than normal depending on the angle of the projected ray. The abscissa 0 is the standard line and along it are measured the degree of rotation. The distance from the ordinate y to π is 180 degrees. The distance from the ordinate Y to φ is the number of degrees the object must turn to corss the central ray. The latter is represented by the "standard line" (Fig. 6). This angle φ for a point.

Law—2. If the object is a point (Fig. 6) the continuous rotatogram is represented by the formula $y=r \sin\frac{a+b}{a+\gamma \cos\theta}$ where r is the point to rotation center distance, a is the tube focus to rotation center distance and b is the rotation center to film distance. When the curved line in the rotatogram formed by the projected image of the point object is at $\theta=\cos^{-1}\frac{r}{a}$ that line has the maximum value of $r\ (a+b)\frac{\sqrt{a^2-r^2}}{a^2+r^2}$. When a approaches infinity this curve becomes a sine curve and the maximum value will be r.

Law—3. If the rotated object is a straight line (Fig. 7 A) this line is inclined to the central line by angle φ at the beginning of rotation. The

image on the rotatogram is a curved zone of variable width which converges at one point. At this degree of rotation the line is parallel to the part of the X-ray beam in which it lies. The degrees of rotation measured along abscissa θ from ordinate y to this convergent point is angle φ for a straight line. The density of the curved zone is variable and inversely proportional to the width.

Law—4. If the rotated object is a solid with one concave surface there is an imaginary line bridging the concavity (Fig. 7 B). This imaginary line is inclined to the central line by an angle φ at the beginning of rotation. The image on the rotatogram is a curved zone of variable width. At one point on the edge of this zone two lines overlap (C on Fig. 7 B). This overlapping point differs from that of a straight line in that there is no progressive increase in the density of the shadow. This is called a rodal point.

Law—5. If the rotated object is a solid with a convex surface the image on the rotatogram is a curved zone with smooth edges and the width is variable unless the cross section of the object is a circle (Fig. 8 A).

Law—6. If two rotated objects exist independently the image on the rotatogram comprises two curved zones which at some point, however small, are completely separate from each other (Fig. 9 A). If one of two rotated objects is completely within the other F(ig. 9 B) the image in the rotatogram is two curved zones one of which is contained entirely within the other.

Several generalizations can be made from these laws. The rotatograph image is larger than the original object but if the target-film distance is sufficiently great, as in teleroentgenography, this error is small (Law 2). If the rotatograph image is a curved line the original object is a point and if the image is a curved zone the original object is a plane or solid. If the sides of the zone of the solid are sharp, smooth and continuous the contour of the cross section has a convex curved perimeter (Law 5). If the image is bounded by dense lines the object is hollow.

To determine the position of a point in a rotatogram the polar coordinates are used (Fig. 6 B). Select a location on the curved line of the rotatogram that is the greatest distance from the standard line as measured along a line parallel to the y coordinate. Draw a circle with this distance as the radius. Draw a line horizontally through the center of this circle, the central line. This line represents the central ray of the x-ray beam with the x-ray tube to the left of the circle. Since the exposure starts and stops at the beginning and end of the 180 degrees rotation the length of the θ abscissa or standard line minus the width of the slit in the cassette tunnel diaphragm (corrected θ length) is 180 degrees. If the film movement has been proportional to the degree of rotation, that is, the

film moving a definite and constant number of millimeters for each degree of object rotation then one degree on the rotatogram is $\frac{1}{180}$th of the corrected θ abscissa length. Alon the abscissa measure angle φ according to Law 1. In the circle already drawn draw a radius inclined to the central line by this angle measured counter to the direction of object rotation °Fig. 6) Where this radius intersects the circle is the location of the point.

To locate the position and limits of an object larger than a point a similar method is used by determining the position of two points. Following the description already given for a single point mark on each side of the curved zones on the rotatogram the point that is the greatest distance from the θ abscissa (r1 & r2, Fig. 8). Draw two concentric circles using these distances as radii. Determine angle φ for each side of the solid by measuring the angle φ_1 to where the object's side first crosses the standard line and angle φ_2 to where the other side crosses it. Use these angles to draw radii in the concentric circles as described for a simgle point location. This gives a pie shaped segment as in Fig. 8 in which the object position is bounded by the radii and the enclosed arcs of circles.

Direct crossgraphy.

Crossgraphy produces a roentgen shadow image of a cross section of any body part at any given level. Immediately after exposure and development the crossgram is ready for interpretation without additional mathematical or instrumental manipulation providing a method of more general clinical application than the other methods described here. The image size is slightly enlarged due to the divergent beam as in conventional roentgenograms. Best results are obtained where there is little motion of the body part. On such films the detail of the cross section is easily seen. Body parts, such as the chest, which have motion between exposures tend to have detail that is less distinct.

Indirect crossgraphy.

Indirect crossgraphy gives a cross section image having a noraml size and shape. To produce such an image a continuous rotatogram is made first. This film (Rg) is placed in the sliding film holder of the apparatus illustrated and described in Fig. 11 in the same position in which it was taken assuming that the tube focus is at F. The room is darkened and a sensitive plate (not film) is placed on the rotating table. The projector light is turned on. At the same instant the table begins to rotate and the rotatogram moves upward simultaneously so that the table

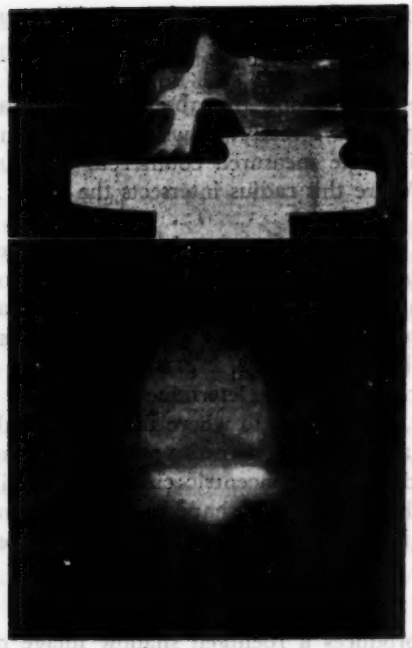

Fig. 10. A. A vertebra mounted on a rotating table in position for direct corssgraphy. The white line indicates the level to be sectioned.
B. A direct crossgram of the vertebra alone at the level indicated.

rotates 180 degrees in the same time that the 180 degrees represented on the rotatogram passes behind the slit S. The processed plate has a cross section image of same size and shape as the original object (Fig. 12).

Discussion.

The methods of roentgenographic examination just described provide a means of examining the appearance and relationship of structures in a cross section of any body part or level in a more direct fashion than the more indirect conventional procedures usually employed. As an example the shadows of a round and a square rod appear essentially the same when viewed from the side and only with some difficulty can one arrive at a reasonably accurate conception of the contours by the customary techniques. A cross section examination reveals these contours readily.

These methods have certain advantages and disadvantages. Discontinuous rotatography produces an image in which the size and position of structures are normal although the outlines are slightly angular and the

Fig. 11

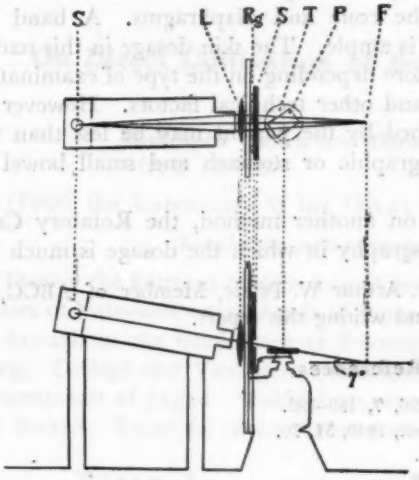

Fig. 12

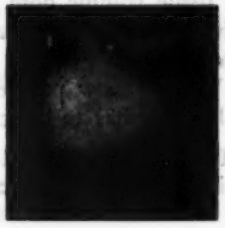

Fig. 11. A schematic drawing of the apparatus used to convert a continuous rotatogram to an indirect crossgram. On rotating table T is placed a sensitive plate P (not film). Vertical light tight shield S contains a transverse slit 4 mm wide. Behind this the rotatogram R*g* is placed in a sliding holder movable in a vertical direction. Condensing lens L brings the lamp S to focus at F. In aligning the system the optical axis is made 7 degrees to the table top. The lamp filament is then brought to focus. Distance F to rotation table center is made equal to the tube focus.rotation table center distance and the film to rotation table center distance is also the same as when the rotatogram was made. If the rotatogram is large it may be reduced photographically and the above distances reduced proportionately.

Fig. 12 Indirect crossgram of the r. human Forearm in the lower part.

process is laborious and time consuming.

Continuous rotatography produces an image that gives good relationship of parts but also requires mathematical and geometrical computations to acquire this information. The use of the projection apparatus to produce an indirect crossgram gives a photographic image with gradation of tonal values as in conventional radiographs with the image normal in size. This additional procedure requires time and more apparatus.

Direct crossgraphy yields a radiographic image that can be studied immediately after exposure and development. Certain distortions occur. Due to divergence of the x-ray beam the parts are slightly enlarged as in conventional radiographs. The contour of some parts is changed such as round outlines assuming a slight oval shape if they are some distance from the center of rotation. These changes are slight and offer little difficulty in interpretation so that from the practical clinical stand point the direct crossgraphy is the most useful of the methods described.

Since the exposures are long it is important to collimate the x-ray beam using narrow slits in the cone and diaphragms. A band one centimeter wide on the patient is ample. The skin dosage in this narrow band may be 50 r or possibly more depending on the type of examination, sensitivity of screens and film and other technical factors. However the total tissue volume dose absorbed by the patient may be less than that received during the usual urographic or stomach and small bowel examination.

Work is now progressing on another method, the Rotatory Crossgraphy,[4] similar to direct crossgraphy in which the dosage is much less.

The writer is indebted to Dr. Arthur W. Pryde, Member of ABCC, for his kindly help in constructing and writing this report.

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On Direct Cultivation of Bacterium Tularense.

By

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(Received for publication, April 16, 1951)

During the fourteen months from November, 1949 to February, 1951, 53 cases of Yato-byo, or tularemia of Japan, were reported to have broken out throughout the whole area of Fukushima Prefecture and in parts of Miyagi, Tochigi and Yamagata Prefectures, all of which are situated in the north-east of Japan. Table I shows the number of the patients in each district; Table II shows the frequency of each clinical type.

TABLE I.

Number of Cases in North-east of Japan during 14 Months Ending February, 1951.

Name of prefecture	Number of cases
Fukushima	39
Miyagi	10
Tochigi	3
Yamagata	1
Total	53

TABLE II.

Frequency of Clinical Types.

Clinical types	Number of cases
Ulceroglandular	11
Oculoglandular	3
Glandular	39
Typhoidal	0
Total	53

40 cases out of all were treated surgically with the total removal of the pathologic lymph nodes; in 6 cases of which streptomycin was given to the patients in combination with surgical operation, with the fairly satisfactory result of their recovery in about two weeks.

We have tried to cultivate directly the Bacterium tularense taken from the lymph nodes of the above forty patients.

The following is to make clear that the isolation of the organism from man has become easier in this country than it was before.

Materials and Methods.

Patients: 39 cases were Glandular or Ulceroglandular; only one was Oculoglandular. 10 out of them were treated in other hospitals and their removed lymph nodes, with blood, were sent to us for examination.

Contact with wild rabbits (Japanese hares and Echigo hares) was, with no exception, seen in the histories of all. Diagnosis was based mainly on the clinical symptoms; in our hospital, intradermal test with phenolised 0.01% bacterial suspension, as well as serum agglutination and slide agglutination, was employed.

Appearance of materials and their clinical examination: The materials used were small pieces of tissue punctate or pus of the axillary, elbow or cervical nodes excised 3 to 48 days after the onsets, except one case in which the interval was about two months. They contained multiple grayish and yellow-white necrotic foci partly abscessed. The pus was highly mucous and our simple staining could not prove the existence of pyogenic cocci in it; however, minute cocci of a sort, less than 0.5 μ in diameter and negative to Gram's staining, were recognizable.

In several cases we studied the motility of the minute cocci in the pus by dark-field examination and could observe their peculiar motion among leucocytes. As a control material we used pus taken from five cases of acute lymphadenitis, in which staphylococci showed the unmistakable Brownian motion. The former's characteritic motion differed markedly from the latter's Brownian motion.

Methods: Two kinds of solid media were used. One was coagulated hen's egg-yolk medium which has been popularly known to be the best medium obtainable for first isolation of Bacterium tularense from pathologic sources. The other was pig-liver medium (Hiwatashi); it is an improved blood-glucose-cystine-agar (Francis).

The former was a mixture of 40% sterile saline solution and 60% pure egg-yolk, coagulated.

To get the latter, a filtered extract was made from pig-liver cut into small pieces and mashed, and this extract served as sustitute for beef infusion in getting blood-glucose-cystine-agar by ordinary procedure.

The materials were rubbed onto these media immediately, as a rule, after operation. When the materials were those sent from other hospitals, the process had to be delayed by 24 to 72 hours after that. The temperature in the incubator was 34°C.

Results of Experiments.

As shown in Table III, when yolk medium was used, direct isolation of the organism was successful in 9 out of 20 cases.

Compared with this, however, a far better result was reached through the use of pig-liver medium, where 14 out of 20 cases turned out to be successful.

Much has already been discussed as to cultivation on the former

TABLE III.

Results of Direct Cultivation.

Kind of medium	Number of tested cases	Number of successful cases
Egg-yolk	20	9
Pig-liver	20	14
Total	40	23

medium, so here our statement will naturally be restricted to what we have discovered on the latter.

48 hours were the minimum necessary time before the growth started; in some cases where the growth was slow, it took as long as 7 days for the first colonies to be formed. The colonies, small and circular at first, were seen, like drops of water, to stand isolated one time and become confluent another, coming finally to take on a slightly bluish, opalescent tinge. When a small bit of the culture was taken out with a loop and shaken on a slide in a drop of saline solution, it became colloidal and mucous.

All these cultivated germs had a conspicuous tendency to agglutinate with the patients' serum.

Getting subcultures on the pig-liver medium was possible even after three months' lapse of time at the room temperature of about 28°C in summer. It usually took 24 hours for the subculture germs to start growth.

Virulence of the organism: A 750 g. guinea pig died nine days after it had been subcutaneously inoculated with 0.002 mg. of the oldest strain (Ebina) of 48-hour cultivation. From its pathologic liver the same organism could be detected by cultivation.

Morphological features: Our observation on the morphology and motility of the germ in question is in process at present; we are using those fourteen strains (No. 1 to No. 14) listed in Table IV as well as seven others cultivated on egg-yolk medium and the heart blood of infected mice.

Its motility is being studied by dark-field examination in saline solution. The study of its morphological structure is being done through the simple staining (carbolic gentian violet), Gram's, and the flagella staining (Saizawa-Sugawara's, Kobayashi's, and Gray's) method.

At the prsent stage of our study, we have succeeded only in recognizing that the organism from these Yato-byo strains reveals polymorphism of high degree, its forms changing with the changes in the cultivating conditions, and that certain forms of the cultivated organisms and of those in the heart blood of infected mice occasionally reveal an obvious active motion which cannot be found in the case of the organisms in human pus.

On the six cases in which cultivation was unsuccessful: We failed to isolate

TABLE IV.

Results of Cultivation by Pig-liver Medium.

No.	Designation of culture	Pathologic source	Interval between onset and test	Days required before formation of first colonies	Agglutination title
1	Ebinr	tissue and pus	20 days	4	1280
2	Oura	tissue and pus	20 "	3	640
3	Nagasawa	tissue and pus	20 "	7	40
4	Wataume	tissue and pus	10 "	5	160
5	Momma	tissue and pus	8 "	6	80
6	Orikasa	punctate and tissue	13 "	2	160
7	Shiraiwa	tissue	3 "	3	(−)
8	Kusakari	punctate and tissue	4 "	4	(−)
9	Yamada	tissue and pus	18 "	3	80
10	Namatame	punctate	16 "	7	1280
11	Kaneko	tissue and punctate	10 "	5	(−)
12	Samago	tissue	9 "	4	(−)
13	Sayu	tissue	9 "	3	(−)
14	Takano	tissue	11 "	5	160
15	Saharu	punctate (pus)	48 "	—	80
16	Kanna	punctate (pus) and tissue	48 "	—	80
17	Komatsu	punctate (pus)	30 "	—	160
18	Watatome	tissue	ca. one month	—	80
19	Oka	tissue	44 days	—	320
20	Satatsu	tissue	ca. two months	—	80

directly the organism in six cases where the material was human pus in three and tissue of lymph nodes in the other cases. In all of these the intervals between the onsets and cultivations were about a month or more, during which period the patients were given such drugs as penicillin, sulfonamides, antipyretics, etc.

In one of these cases the patient experienced no other subjective symptoms than adenopathy in both axillary fossae, which was found, even after a month, to contain many necrotic foci but showing no sign of suppuration.

We also tried to isolate the germ through inoculated mice and failed. A possible explanation for our failure to isolate it may be that, in these unsuccessful cases, the organisms in the examined materials were not strong enough or utterly dead.

SUMMARY.

Since McCoy and Chopin succeeded in 1912 in isolating Bacterium

tularense using egg-yolk as medium, many contributions have been made towards securing better media for cultivating this organism. Among them, Foshay[1)2)] and his associates, Gibby, Nicholes and Tamura[3)], succeeded recently in getting more suitable liquid media. They are at present making further efforts to elucidate the effects of the components of the media upon the growths.

In this country, though some amount of contribution has been made in this field of medicine, it is still difficult to directly cultivate Bacterium tularense from any given pathologic source, and egg-yolk has been considered to be the most suitable medium for this purpose.

Pig-liver medium was devised by Hiwatashi a short time ago and has hitherto been valued as suitable for subculture; but, as the one to be used for first isolation of the organisms got from human pathologic materials, it has not been given much credit.

T. Ota,[4)] after an attempt to isolate Bacterium tularense using the Hiwatashi medium in nine cases out of more than forty of tularemia which occurred 1949 in Chiba Prefecture, had to confess his failure.

In the latest outbreak of Yato-byo in this part of Japan, we at first used egg-yolk medium for direct cultivation. But, since the spring of 1950, we have come to find a far better medium in pig-liver. It certainly enables us to make further study of the morphology of the organism more easily, of which the present work is only a part.

And it may well be stated that the direct isolation of Bacterium tularense from human lymph nodes presents a considerably good result and has its diagnostical value in case the materials can be secured within about a month after onsets and inoculated onto suitable media; though it will be difficult to do so after the lapse of over a month of the clinical course, even if animal examination is performed immediately after operation.

We express our deepest gratitude to Prof. T. S. Katsura, of Tohoku University, and to Dr. K. Sato, Director of Fukushima Branch Office of the Laboratory for Infectious Diseases, for their valuable suggestions and guidance in preparing this. Thanks are also due to Dr. Honda, Dr. Sase and Dr. Shiraiwa for their kindly providing us with the materials.

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Studies on the Antibiotic Substances from Actinomyces.

(14th Report)

In Vitro and In Vivo Activities of Several Kinds of Basic Antibiotics from Actinomycés upon Pasteurella Tularensis.

By

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(Received for publication, June 11, 1951)

The activities of several kinds of basic antibiotics from actinomyces isolated in this laboratory were tested upon *Pasteurella tularensis* in vitro and in vivo, being compared with those of streptomycin, chloromycetin and colistin and the results obtained were as follows:

1) The in vitro activities of these substances upon *Pasteurella tularensis* were arranged in the following order:

Streptomycin (3 μg), Roseomycin (25 μg), No O-20 (50 μg).

Minimal inhibitory doeses were indicated too. All other substances, i. e. chloromycetin, colistin, No 259 and Seki substances showed no activities at all.

2) In experimental tularemia infection in mice, both streptomycin and roseomycin showed a similar excellent preventive action in a same dose (1 mg per day divided into 4 doses) in spite of different activities in vitro.

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Adrenaline Load of the Suprarenal Glands of Rabbits in Histamine Poisoning.

By

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(Received for publication, April 21, 1951)

On the glycemic action of histamine, an interesting fact was found by Satow[1] in our laboratory. In rabbits, hyperglycemia is caused by the intravenous injection of histamine in a dose of 0.4–0.5 mg per kg rapidly (in 5–20 seconds). On the contrary, blood sugar decreases, when the same dose of histamine is injected slowly (in 1–1.5 minutes). This was confirmed in rabbits also by Takita[2] in our laboratory. Therefore it seemed to be of interest to know how acts the histamine, adminstrated rapidly or slowly, on the adrenaline load of the suprarenals.

As regards the influence of histamine poisoning upon the adrenaline load of the suprarenal glands some papers[3] dealt with.

Method.

Healthy rabbits were used. Histamine phosphate ("Takeda") was injected in a following way.

(a) 0.5 mg histamine per kg of body weight was injected intravenously as a 0.1% solution in 10 seconds.

(b) Histamine in a same dose was injected intravenously in 60 seconds.

(c) 20 mg histamine per kg was injected subcutaneously as a 1% solution.

In some cases the animal died after histamine injection. In these cases the suprarenal glands were extirpated soon after the death. In other cases, 30 minutes after intravenous injection or 120 minutes after the subcutaneous injection, after killing the animal by a blow on a neck, the surparenals were removed. For the estimation of adrenaline in the suprarenals, the corrosive sublimate method of Suto-Kojima[4] was used, taking Adrenaline chloride ("Sankyo") as a standard. The actual concentration of adrenaline was determined by the method of Kodama.[5]

Results.

The adrenaline load of suprarenal glands in 9 normal rabbits was determined as a control of the present investigations. The results were

TABLE I.

Adrenaline Load in Suprarenal Glands of Normal Rabbits.

No.	Date	Body weight (kg) and sex	Weight of suprarenal gland (g)		Adrenaline load (mg)					
					total quantity		per g gland		per kg of body weight	
			R	L	R	L	R	L	R	L
1	2. X. '42	1.53♀	0.115	0.126	0.075	0.071	0.65	0.56	0.049	0.046
2	7. X. '42	2.11♂	0.177	0.198	0.071	0.074	0.40	0.37	0.034	0.035
3	22. X. '42	1.73♀	0.080	0.104	0.043	0.045	0.54	0.43	0.025	0.026
4	12. XI. '42	2.56♂	0.385	0.385	0.126	0.109	0.33	0.28	0.049	0.043
5	29. IX. '43	1.74♂	0.164	0.166	0.067	0.066	0.41	0.40	0.039	0.038
6	39. IX. '43	1.90♀	0.276	0.281	0.111	0.120	0.40	0.43	0.058	0.063
7	12. X. '43	1.65♀	0.091	0.104	0.061	0.063	0.67	0.61	0.037	0.038
8	22. XII. '43	1.73♀	0.109	0.124	0.072	0.068	0.66	0.55	0.042	0.039
9	25. XII. '43	1.26♀	0.103	0.109	0.050	0.050	0.49	0.46	0.040	0.040
Average		**1.80**	**0.167**	**0.177**	**0.075**	**0.074**	**0.51**	**0.45**	**0.041**	**0.041**

displayed in Tab. I. In these normal rabbits the adrenaline load of suprarenals per gland was in an average value 0.075 mg for the right gland and 0.074 mg for the left gland, the adrenaline load per kg of body weight was in a mean 0.041 mg for the suprarenals of each sides and adrenaline per g of gland was in a mean value 0.51 mg for the right gland and 0.45 mg for the left gland. These values coincide nearly with the value reported in the statistical studies of H. Sato on the adrenaline load of suprarenal glands of 617 normal rabbits, only the adrenaline load per g of gland of the present experiments is somewhat smaller than that of H. Sato.

The chief experiments were performed on 23 rabbits. The results were presented in Tab. II.

In 8 rabbits, histamine in a dose of 0.5 mg per kg of body weight was injected intravenously in 10 seconds. Soon after the injection the intensive convulsions took place in 4 rabbits, and 3 rabbits out of them died eventually. In other 4 rabbits the convulsions did not occur, and only the tachycardia and depression were observed. In averaging the values in these 8 rabbits, the adrenaline load per gland was 0.080 mg for the right gland and 0.079 mg for the left gland, adrenaline per kg of body weight was 0.043 mg for the suprarenals of each sides, and adrenaline per g of gland was 0.49 mg for the right suprarenals and 0.44 mg for the left suprarenals.

In other 8 rabbits the same dose of histamine was injected intravenously in 60 seconds. Only one rabbit out of them died after a occurrence of intensive convulsions. In other rabbits the convulsions were

Table II.

Adrenaline Load in Suprarenal Glands of Rabbits in Histamine Poisoning.

No.	Date	Body weight and sex	Weight of suprarenal gland (g)		Adrenaline load (mg)						Duration after injection to death (†) or slaughter (min.)
					total quantity		per g gland		per kg of body weight		
			R	L	R	L	R	L	R	L	
	(A) Rapid intravenous injection of histamine (0.5 mg per kg). Duration of injection 10 sec.										
1	14. IX. '42	2.53 ♂	0.296	0.297	0.074	0.070	0.25	0.24	0.029	0.028	30
2	18. IX. '42	1.67 ♂	0.199	0.174	0.094	0.083	0.47	0.48	0.056	0.050	30
3	18. IX. '42	2.06 ♂	0.182	0.184	0.054	0.050	0.30	0.27	0.026	0.024	6†
4	21. IX. '42	1.86 ♀	0.107	0.124	0.117	0.113	0.99	0.91	0.063	0.061	30
5	21. IX. '42	1.61 ♂	0.230	0.245	0.111	0.114	0.48	0.47	0.069	0.071	4†
6	25. IX. '42	1.58 ♂	0.118	0.131	0.091	0.093	0.77	0.71	0.058	0.059	30
7	28. IX. '42	1.83 ♀	0.119	0.155	0.034	0.040	0.29	0.26	0.019	0.022	5†
8	28. IX. '42	2.45 ♂	0.276	0.313	0.063	0.065	0.23	0.21	0.026	0.027	30
Average		**1.95**	**0.191**	**0.203**	**0.080**	**0.079**	**0.49**	**0.44**	**0.043**	**0.043**	
	(B) Slow intravenous injection of histamine (0.5 mg per kg). Duration of injection 60 sec.										
9	11. XII.'43	1.53 ♂	0.111	0.166	0.073	0.065	0.66	0.39	0.048	0.043	30
10	11. XII.'43	1.70 ♂	0.272	0.282	0.059	0.065	0.22	0.23	0.035	0.038	30
11	12. XII.'43	2.06 ♂	0.276	0.278	0.084	0.091	0.30	0.33	0.041	0.044	30
12	12. XII.'43	2.15 ♀	0.224	0.277	0.068	0.077	0.30	0.28	0.032	0.036	6†
13	22. XII.'43	1.74 ♂	0.189	0.250	0.151	0.155	0.80	0.62	0.087	0.089	30
14	24. XII.'43	1.60 ♀	0.110	0.114	0.057	0.055	0.52	0.48	0.036	0.034	30
15	24. XII.'43	1.62 ♀	0.098	0.093	0.054	0.050	0.55	0.54	0.033	0.031	30
16	25. XII.'43	1.90 ♂	0.172	0.213	0.074	0.056	0.43	0.26	0.039	0.030	30
Average		**1.79**	**0.182**	**0.209**	**0.078**	**0.077**	**0.47**	**0.39**	**0.044**	**0.043**	
	(C) Subcutaneous administration of histamine (20 mg per kg).										
17	25. IX. '42	1.66 ♀	0.105	0.101	0.052	0.050	0.50	0.50	0.031	0.030	120
18	22. X. '42	1.81 ♀	0.134	0.136	0.034	0.027	0.25	0.20	0.019	0.015	120
19	29. X. '42	1.77 ♂	0.116	0.126	0.062	0.061	0.53	0.48	0.035	0.034	120
20	7. X. '43	1.84 ♀	0.141	0.174	0.069	0.067	0.49	0.39	0.038	0.036	120
21	12. X. '43	1.91 ♀	0.271	0.302	0.075	0.071	0.28	0.24	0.039	0.037	120
22	18. XII.'43	2.18 ♂	0.263	0.331	0.061	0.072	0.23	0.22	0.028	0.033	120
23	25. XII.'43	1.93 ♂	0.285	0.322	0.070	0.067	0.25	0.21	0.036	0.035	120
Average		**1.87**	**0.188**	**0.213**	**0.060**	**0.059**	**0.36**	**0.32**	**0.032**	**0.031**	

not observed. The rate of heart beat increased definitely. The mean value of the adrenaline load of the suprarenals in these rabbits was 0.078 mg and 0.077 mg per gland for the right and the left glands respectively; and 0.044 mg per kg of body weight for the right gland and 0.043 mg per kg for the left gland, and 0.47 mg per g of gland for the right suprarenals and 0.39 mg for the left.

Thus in our experiments non-effect of histamine was observed, which was injected rapidly or slowly in a dose of 0.5 mg per kg upon the adrenaline load of the suprarenal glands.

In another series of experiments, histamine was injected subcutaneously in a dose of 20 mg per kg in 7 rabbits. This dose of histamine is, according to the report of Hirano[7] in our laboratory, about double as much as the maximum non-lethal dose in rabbits and in fact 3 rabbits out of 5 in his experiment died after subcutaneous injection of histamine in a dose of 20 mg per kg. Nevertheless in the present experiments no rabbit died after application of the same dose of histamine. The rate of heart beat increased markedly. The depression and urination were often observed. The adrenaline load of the surparenal glands in these 7 rabbits was on an average 0.060 mg for the right gland and 0.059 mg for the left gland. Reckoning the body weight or the weight of gland, they are in a mean value 0.032 mg per kg of body weight in the right gland and 0.031mg per kg in the left gland, 0.36 mg per g of gland in the right gland and 0.32 mg per g of gland in the left gland.

Comparing these values with those of the control experiments, it is clear that the subcutaneous application of histamine in a dose of 20 mg per kg of body weight causes a reduction of adrenaline load in the suprarenal gland.

In this connection we should recall the report of Z. Kanowoka[8] on the peptone poisoning. When the animal does not die within a short period and the symptoms of poisoning continue for a certain length of time, the adrenaline load decreases. On the other hand, when the poisoning symptom is severe and the animal dies in a short period, adrenaline load of the suprarenals is not reduced.

Summary.

From the evidence reported by Satow that the glycemic action of histamine is quite different in using the different manner of applications, the interest was evoked, and the present studies were undertaken to know whether the similar evidence can be found or not concerning the influence of histamine upon the adrenaline load of the suprarenal gland.

Rabbits were used. The adrenaline load of the suprarenal glands was estimated by the corrosive sublimate method of Suto-Kojima. His-

tamine was injected intravenously in a dose of 0.5 mg per kg of body weight in 10 seconds or in 60 seconds. Some rabbits died several minutes after histamine injection and others were able to recover. In the former the suprarenals were removed soon after death and in the latter it was removed 30 minutes after injection. In these experiments we could not find any definite alteration of adrenaline load after histamine. Subcutaneous injection of histamine in a dose of 20 mg per kg was also applied. In this series of experiments the reduction of adrenaline load was observed.

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A Statistics about the Causes of Delay in the Diagnosis of Stomach Cancer.

By

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All stomach cancer would be curable, if the diagnosis were made early enough and the proper treatment were applied immediately, however, yet it is said that the prognoisis of the stomach cancer is serious. They say the reason of this is due to the delay between the time when the patient has first symptoms and the time when the definitive treatment begins. Concerning this, we investigated 652 cases of stomach cancer in ten years between 1940 and 1949 in our medical clinic, and we could get the following results:

1. In 77, 1% of 652 cases there was an undue delay in making the diagnosis, and in 22,9% of them there was no delay. The delay in the diagnosis, during ten years in 1940–1949, had decreased from 80,3 to 70,3%. "No delay" had increased from 19,7 to 29,7%.

2. The responsibility for the delay of dignosis from side of the patient was 32,9% and that from side of the physician was 35,3%. The percentage of delay by the patient had decreased from 40,9 to 24,4% in these ten years.

3. In 64,5% of 287 cases, whose delay in making the diagnosis were caused from side of physicians, physicians failed to arrive at a diagnosis in one month or, if unable to do so, did not refer the patient to a suitable person, hospital or clinic. In 26,8% of them had wrong treatments.

4. In average of 652 cases of stomach cancer 6,07 months from first symptoms to the start of proper treatment were spent in vain and the term which is due to the patients responsibility is 3,26 months from side of physician. The period of which the responsibility owes to the patient had decreased gradually from 3,35 to 2,95 months in these ten years. However, it has no change from the side of the physician.

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A Note on the Estimation of Adrenaline in the the General Blood.*

By

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(Received for publication, April 21, 1951)

Many attempts were made to seek the suitable method for the estimation of adrenaline in the general blood. Nevertheless the adrenaline content therein is too small to estimate it by the commonly used methods. More senstive and specific methods are desirable. In this connection we recall the methylene blue method.

The methylene blue method, which was devised by Euler[1] for the quantitative determination of adrenaline is based on the fact reported by Ahlgren,[2] that the decolouration of methylene blue by finely divised muscle tissue is accelerated by adrenaline in concentration between 10^{-15}-10^{-17}. According to Euler, the serum of normal peripheral blood in human beings or in rabbits in concentration of about 1:200—1:400 accelerates the decolouration of methylene blue. He claimed that the accelaration by sera will be due to the adrenaline in the sera, as the sera of rabbits after removal of suprarenal glands on both sides are less effective than before suprarenalectomy. It was thus calculated by Euler, that the adrenaline in peripheral blood of human beings or of rabbits is about 10^{-13}–$10^{-12.5}$. This method was used by several workers. (Euler and Liljestrand,[3] Euler and Holmquist,[4] Bross and Kubikowski.[5])

Now the present investigation was undertaken to ascertain its availability.

Experimental.

I.

As the control experiment in methylene blue method, muscle tissue of frog was suspended in a methylene blue solution in some Thunberg's tubes without adding either adrenaline or serum solution, and the methyl-

* All of the experiments in the present investigation were performed during April, 1941—June, 1942.

ene blue decolouration time was estimated in every tube.

0.20 g of hind limb muscle of frog (rana fusca japonica or rana nigromaculata), which was divided finely with small scissors, was suspended in the following solution (0.9 cc) in every tube. 5 cc of 1:500 methylene blue solution (Methylenblau medicinale "Bayer") +5 cc of 1/10 m K_2HPO_4+5 cc of re-distilled water. The tube was evacuated in pressure of about 3 mm Hg. The pressure of 10 mm Hg, which was described in the papers of Ahlgren and of Euler, is not sufficient, as the methylene blue is decolourised very slowly in the tube evacuated at a pressure such as 10 mm Hg. Just after the evacuation, the tube was immersed in a thermostat (35°C.) and the time till the decolouration of methylene blue was noted.

The maximum difference of decolouration time among each tube was in the most of our experiments about 10% of the decolouration time. Some of the experimental results are given in following table.

No. of tubes	1	2	3	4	5
Decolouration time (min.)	31	29	30	28	29

No. of tubes	1	2	3	4	5	6	7	8	9	10	11
Decolouration time (min.)	17	17	18	16	17	18	17	16	18	18	17

In the paper of Ahlgren, it was showed that the methylene blue decolouration time was constant and its maximum difference was one minute. In a paper of Euler and Holmquist, the maximum difference was 7% of decolouration time. On the contrary, Nagae[6] claimed that the difference of decolouration time such as 3–5 minutes was very often observed. Although our experimental results were not so divergent as that of Nagae, it may be said from our results that the difference of decolouration time such as smaller than 2 minutes laid within the limits of an experimental error.

In the next place, the effect of adrenaline on the decolouration of methylene blue was studied. Adrenaline chloride (Sankyo Co.) was used and its actual concentration was estimated by Kodama's[7] modification of Folin, Cannon and Denis's method. 0.1 cc adrenaline solution in different concentrations was added to the tubes in which 0.20 g muscle tissue and 0.9 cc suspension solution were contained. In the control tubes 0.1 cc re-distilled water was added instead of adrenaline solution. In this series of experiments, the results are not so constant.

Very often was observed non-effect of adrenaline as shown in the following table.

No. of tubes	1	2	3	4	5	6	7	8	9
Adrenaline	—	—	10^{-14}	10^{-13}	10^{-12}	10^{-11}	10^{-10}	10^{-7}	—
Decolouration time (min.)	14 (control)	15 (control)	14	16	15	14	15	13	14 (control)

However, in some experiments, adrenaline in the concentration of 10^{-12}–10^{-9} accelerated the decolouration of methylene blue. Such a result is given in following table.

No. of tubes	1	2	3	4	5	6	7	8	9
Adrenaline	—	10^{-10}	10^{-11}	—	10^{-13}	10^{-14}	10^{-12}	10^{-9}	10^{-5}
Decolouration time (min.)	37 (control)	32	30	38 (control)	37	36	31	34	38

In some other experiments, the retardation of methylene blue decolouration in small degree was observed by adding adrenaline in a concentration of 10^{-13}–10^{-8}.

Such inconstant results as above quoted left us a serious doubt of the availability of this method.

Our experimental results are thus on the one hand quite dissimilar to that of Ahlgren, who reported a very constant data. In his experiment, adrenaline accelerated the methylene blue decolouration in a concentration of about 10^{-15}–10^{-7} and the maximum effect of adrenaline was observed in 10^{-12}–10^{-11}. From the liminal concentration of adrenaline ,10^{-15}, to 10^{-12}–10^{-11}, the effect of adrenaline increased concomitantly with the increase of adrenaline concentration. This was confirmed by Euler. On the other hand, all the reports from the other sources, viz. of Nagae, Tadakiyo Satake[8] and Radsma & Tjhana,[9] failed entirely to duplicate the findings of A. and E.

II.

The serum of the general blood of normal rabbits was diluted in different concentration and 0.1 cc of it was added in each tube in which the muscle tissue was suspended in methylene blue solution. After the tube was evacuated, the decolouration time was measured. The decolouration of methylene blue was somewhat accelerated by adding the serum in a concentration above 1:50. One example in this series of experiments is given in the following table.

Euler observed the decrease of accelerating effect of rabbit's sera after removal of suprarenal gland and from this observation he assumed the accelerating substance in the sera to be adrenaline. To ascertain this point, the experiment in bilaterally suprarenalectomized rabbits were performed. The suprarenal glands were removed through the lumbar

Normal rabbit. ♂ 2.22 *kg*

No. of tube	1	2	3	4	5	6	7	8
Concentration of serum	—	1:25	1:50	1:100	1:100	1:200	—	—
Decolouration time (min.)	16 (control)	12	13	15	14	16	17 (control)	17 (control)

route, at first on the right and next on the left with an interval of some weeks, and the experiments were carried out about 18 weeks after the decapsulation. In our experiments, the decolouration of methylene blue was also accelerated with the sera of the general blood in rabbits after removal of the both suprarenal glands. Between the effect of sera in normal rabbits and that of sera in suprarenalectomized rabbits, we can not notice any definite difference, contrary to the results of Euler. One of the experimental results is shown in the following table.

Doubly suprarenalectomized rabbit ♂ 2.75 *kg*

No. of tubes	1	2	3	4	5	6	7
Concentration of serum	—	1:25	1:50	1:100	1:200	1:100	—
Decolouration time (min.)	26 (control)	19	22	24	27	23	27 (control)

Judging from an example in the paper of Euler, the suprarenals were removed per laparotomiam and his experiment was performed in the next day after the operation. The interval of only one day is not sufficient for the complete recovery from the influence of operation. Euler's result may be rather due to the influence of lacking of the suprarenocortical function than the absence of adrenal medulla.

The experiments were also performed concerning the effect of sera in normal as well as totally sympathectomized dogs on the methylene blue decolouration. Also in this series of experiments we can not observed any definite difference between sera of normal and sympathectomized animals.

Normal dog. ♀ 6.3 *kg*

No. of tubes	1	2	3	4	5	6
Concentration of serum	—	1:25	1:50	1:100	1:200	—
Decolouration time (min.)	39 (control)	30	33	34	37	41 (control)

Totally sympathectomized dog. ♀ 7.8 *kg*

No. of tubes	1	2	3	4	5	6	7	8	9
Concentration of serum	—	—	1:25	1:50	1:100	1:400	—	1:200	1:800
Decolouration time (min.)	24 (control)	24 (control)	17	19	19	25	26 (control)	22	25

From these results we are not justified to accept the view of Euler that the accelerating effect of sera on the methylene blue decolouration by the muscle tissue is due to adrenaline.

Summary.

The present investigation was undertaken to know the methylene blue method is reliable or not for the estimation of adrenaline in the general blood.

The effect of adrenaline on the decolouration of methylene blue by muscle tissue had a large variation. Moreover we failed to find any evidence that the effect of serum of peripheral blood on the methylene blue decolouration is due to adrenaline. In fact, the effect of serum did not differ at all between that from the normal animals and those deprived of the suprarenals or totally sympathectomized.

Therefore the methylene blue method is not available for the estimation of adrenaline in the general blood.

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Our Method of Taking the Radiogram of the Optic Canal.

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There have been reported[1] over 40 kinds of positioning technique in taking the radiogram of the optic canal. It seems to us, however, some of them are too complicated, while others are inconvenient for the practice. For the purpose of taking it more accurately and conveniently, we deviced the following positioning technique.

1). Arrange the X-ray tube and the film so that the central X-ray falls perpendicular to the center of the film which is laid horizontal.

2) Draw a line joining the meatus acusticus externus and the angulus occuli lateralis of the opposite side of the concerned optic canal, and make a triangle with this line as a base. And let the baseangles be 110 degrees at the meatus acusticus, externus and 35 degrees at the angulus occuli lateralis. We call the vertex of this equilateral triangle point A. ($\angle A = 35°$).

3) Next, take a point at the margin of the lower eyelid, 8 mm distant from the angulus occuli lateralis of the same side of the concerned optic canal. We call this, point B.

4) Lay the patient prone on the roentgenographic table and adjust the point B at the center of the film.

5) Adjust then the point A on the vertical line fallen from the tubefocus.

6) Expose.

Under this positioning, 44 cases of healthy female and male were roentgenographed. And we obtained the clear X-ray image of the optic canal in in 42 cases. Only in two cases out of 44 the central X-ray did not hit to the direction of the optic canal.

From the statistical point of view, we conclude then as follows:

When we adopt this positioning technique in the case of roentgenographiing of the optic anal, the confidence limit (possibility in percentage) of missing the optic canal, is caluculated between 7.2 and 6.6 under the confidence coefficient α of 0.05.

I wish to thank prof. Tadao Tanaka for his kind advice with regard to the statistics.

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On the Availability of the Bloor & Bullen's Arseno-Molybdic Acid Method for the Assay of Adrenaline in Suprarenal Vein Blood.*

By

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(Received for publication, April 21, 1951)

For the chemical estimation of adrenaline in blood, Bloor and Bullen[1] reported a more simple and practicable modification of Shaw's[2] arseno-molybdic acid method. In their method, adrenaline in a protein-free filtrate of blood is selectively adsorbed with a specially prepared aluminium hydroxide and by this separated adrenaline the arseno-molybdic acid is reduced to form a blue colour.

In order to know whether Bloor & Bullen's method is available for the estimation of adrenaline in the suprarenal vein blood or not a comparison of adrenaline estimates of the suprarenal vein blood of dog by the Bloor & Bullen's method and by the rabbit intestine segment method was carried out.

Experimental.

The dogs were fastend supine on the table and anesthetized with ether, by dropping it on the towel which covered the nose of animals. The cava pocket was prepared as usual manner. Some suprarenal vein blood specimens were collected with some intervals.

One cc of blood specimen was run into 2 cc of 10% trichloroacetic acid solution just after the collection of blood specimen, well mixed, centrifuged and filtered. One cc of this protein-free filtrate was used for the chemical estimation. The remaining blood of the same sample was used for the adrenaline estimation by the rabbit intestine segment method.

At the end of the collection of the suprarenal vein blood specimens from the cava pocket, arterial blood was collected from the carotid artery, which was used in the estimation by the Bloor & Bullen's chemical method as a control. As the colour due to the arterial blood is given by the alkali-stable adrenaline-like substance and not adrenaline itself, the amount of

* Reported at the XXVI Annual Meeting of the Japanese Physiological Society at Kyoto, 1949, April.

colour corresponded to the arterial blood is subtracted from that due to the specimen.

The procedures used in the chemical determination of adrenaline are essentially those described by Bloor & Bullen. However in order to reduce the colour of the blank, the reagent used was modified slightly. Sodium sulfite solution was prepared by dissolving 2 g of sodium sulfite in 10 cc hot water and was then cooled. Just before use 5 cc of this sodium sulfite solution was added to 30 cc of 1:1 sulphuric acid solution.

15 estimations were performed in 4 dogs. All the results are summarized in Table I. More than a half of all estimations the Bloor & Bullen's

TABLE I.

Adrenaline in the Suprarenal Vein Blood of Dogs Estimated by the Method of Bloor & Bullen and the Rabbit Intestine Segment Method.

No. of animal and date (1948)	No. of specimen	Adrenaline (γ) in 1 cc blood		Ratio Bloor: Intestine
		Bloor	Intestine	
Dog 1 ♂	1	0.32	0.45	1:1.41
2.5 kg.	2	0.56	0.55	1:0.98
28. X.	3	0.49	0.5	1:1.02
	4	0.66	0.7	1:1.06
Dog 2 ♀	1	0.52	0.8	1:1.54
7.2 kg.	2	1.02	1.2	1:1.18
10. XI.	3	0.33	0.35	1:1.06
	4	0.33	0.6	1:1.82
Dog 3 ♂	1	0.19	0.2	1:1.05
9.1 kg.	2	0.44	0.6	1:1.37
17. XI.	3	0.41	0.5	1:1.22
	4	0.45	0.4	1:0.89
Dog 4 ♂	1	0.37	0.35	1:0.95
5.2 kg.	2	0.59	0.5	1:0.85
7. XII.	3	0.8	0.8	1:1.00

chemical method and the rabbit intestine segment method gave values almost identical with each other. But in some cases the chemical method yielded somewhat lower value. The ratio of adrenaline estimates in the suprarenal vein blood determined by the method of Bloor & Bullen and the rabbit intestine segment method was as the mean 1:1.16 (1:0.85-1:1.82). Thus it is derived from our experimental results that the Bloor & Bullen's arseno-molybdic acid method is available for the estimation of adrenaline in the suprarenal vein blood.

SUMMARY.

In etherized dogs, the suprarenal vein blood specimens were collected by the cava-pocket method. Adrenaline content in these blood specimens

was determined by the Bloor & Bullen's arseno-molybdic acid method and the rabbit intestine segment method. An almost the identical value of adrenaline was given by these two methods. But in some cases arseno-molybdic acid method gave a somewhat lower value than the rabbit intestine segment method.

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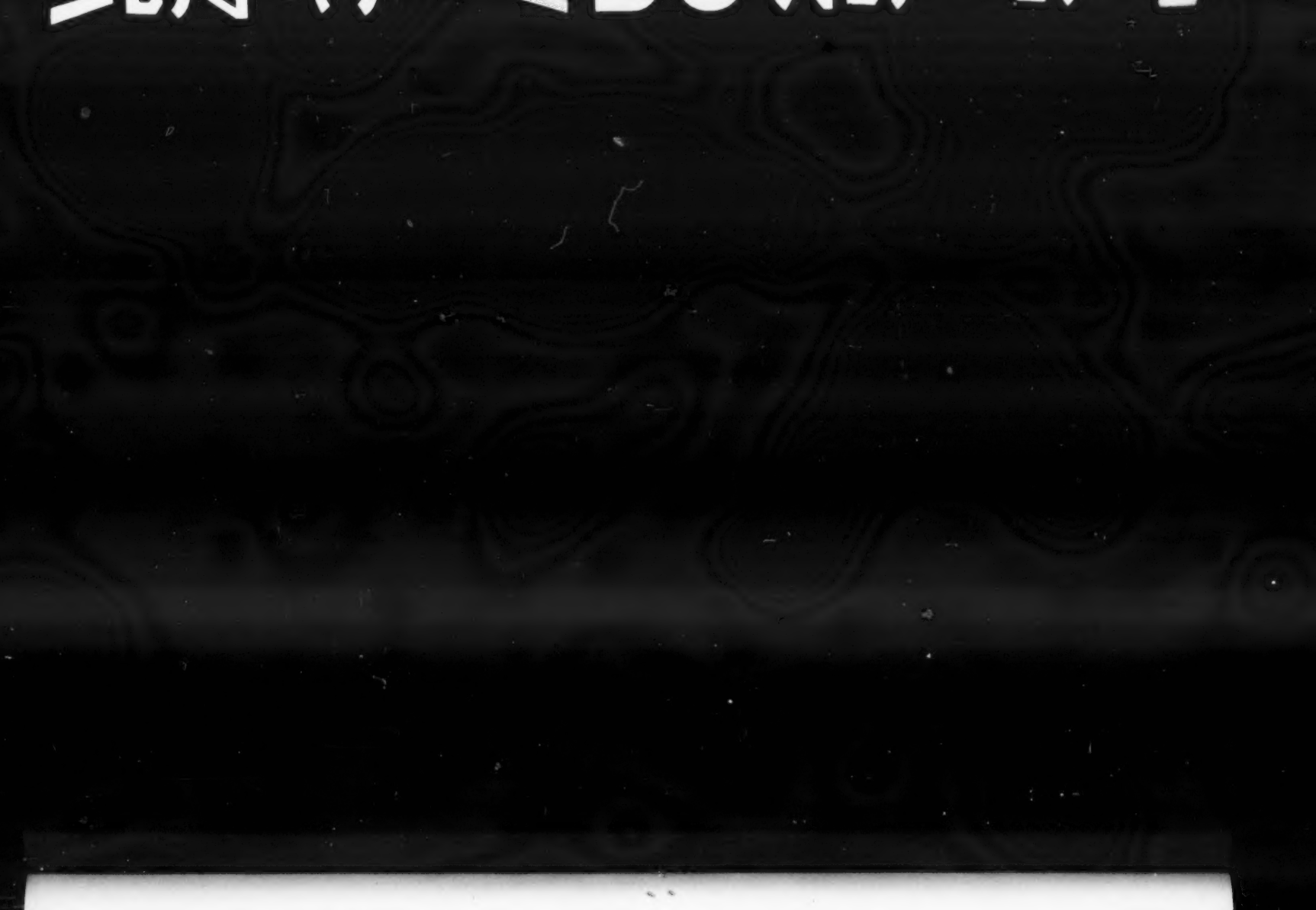
was determined by the Biloe & Bullen's arseno-molybdic acid method and the rabbit intestine segment method. As shown the fibrinal value of adrenaline was given by these two methods. But in some cases arseno-molybdic acid method gave a somewhat lower value than the rabbit intestine segment method.